Plant community impacts and environmental associations of the invasive macroalga *Nitellopsis obtusa* (starry stonewort)

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Abstract

Nitellopsis obtusa (starry stonewort, family Characeae, hereafter *Nitellopsis*) is a large, characean macroalga that is non-native in North America and was first found in Minnesota in 2015. *Nitellopsis* is the first invasive macroalga in the Midwest and arguably the only charophyte considered invasive globally. This novelty has led to high uncertainty of its ecological threat and limited information on best management practices. *Nitellopsis* can spread quickly and grow to dominate littoral zones, generating substantial concern regarding the potential ecological threats that it poses lake ecosystems. I addressed key knowledge gaps on the ecology of *Nitellopsis* by: 1) evaluating impacts of its invasion on native plant communities, and 2) investigating the influence of fine-scale environmental conditions on its growth. To explore these aspects of *Nitellopsis* ecology, I conducted field work using two observational methods – including space-for-time-substitution sampling in 2018 and time sequence sampling from 2017 - 2020. I found that the cover and richness of the native macrophyte community declined as *Nitellopsis* cover increased, and that increases in *Nitellopsis* were further associated with changes to species and functional group composition. While a diverse array of native macrophytes were negatively affected by *Nitellopsis,* the taxa most sensitive to its invasion were *Chara* spp. (characean algae that are native relatives of *Nitellopsis*) and *Najas guadalupensis* (southern naiad), both of which are similar to *Nitellopsis* in having a "carpet-forming" habit. The ability of *Nitellopsis* to invade occupied habitat is indicative of a highly competitive invader. Multi-year observations of *Nitellopsis* invasion revealed that abundance patterns varied between lakes and between years within lakes. Importantly, locations or periods of lower abundance may provide opportunities for

native plants to recover from invasion effects. Abundance of *Nitellopsis* was strongly correlated with its impacts for all native responses evaluated, which was expected and motivated the exploration of *Nitellopsis*' environmental associations. However, the investigation into environmental conditions associated with nuisance growth yielded little explanatory power, except for slight declines in cover of *Nitellopsis* under increasing total nitrogen in sediments. The lack of explanatory power could be due to study limitations or may be indicative of broad environmental tolerances of *Nitellopsis*. The apparent ability of *Nitellopsis* to reach peak growth under a variety of conditions in Minnesota lakes elevates its threat as an invader. Overall, this study revealed that *Nitellopsis* is an aggressive, high-impact invader and spread prevention and management should be prioritized for this species across its invaded range in North America.

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Chapter 1

Introduction: *Nitellopsis obtusa* – An emerging aquatic invader with uncertain impacts

Background

Invasive species can thrive in new environments and negatively affect resident ecological communities and processes. Non-native species invasions are one of the leading causes of biodiversity decline worldwide, especially in freshwater systems, which have the highest species density of any habitat on the globe (Ricciardi and MacIsaac 2011, Thomaz et al. 2015). Freshwater systems, lakes in particular, are among the most sensitive to invasion because of the strong trophic links in aquatic habitats (Gallardo et al. 2016). Aquatic invasions can affect many aspects of an ecosystem – from populations, to communities, to ecosystem processes (Ehrenfeld 2010). These changes may be long-lasting, as eradication is rarely possible once an invader establishes. In the Great Lakes Region alone, there are already over 100 aquatic invasive species (AIS) and that number will likely rise (Escobar et al. 2018a). Research has shown that some of these AIS are highly detrimental to ecosystems and economies, like zebra mussels (*Dreissena polymorpha*) (MacIsaac 1996), while others may have little impact, like the non-native parasite *Heterosporis sutherlandae* (Tommamichel 2018). Some AIS have spread to become nuisances at a landscape scale (e.g., *Potamogeton crispus* [curlyleaf pondweed]), while others are more localized (e.g., *Petromyzon marinus* [sea lamprey] in the Great Lakes) due to limited availability of suitable habitat and/or inadequate prevention efforts.

Managing AIS in the context of high uncertainty regarding the threats they pose and their biology in the invaded range is challenging given the scope of the problem.

Prevention and control of AIS are focal points of lake management efforts. The State of Minnesota alone allocates $10 million in annual aid to counties for AIS prevention and response. The Minnesota DNR further awards funds to lake associations and local units of government for the control of aquatic invasive plants across the state ($530,000 in 2020). Lakes receive high AIS propagule pressure due to human-assisted movement between waterbodies, particularly via boat traffic (Cole et al. 2019). This pathway is particularly problematic for aquatic invasive plants because most reproduce clonally, and can regrow from small fragments that catch easily on trailers, motors, and other equipment (Rothlisberger et al. 2010, Jerde et al. 2012). While significant resources are invested in prevention (e.g., public access inspections), problems associated with AIS continue to grow. They are rarely eradicated once established in a waterbody and require ongoing management to mitigate ecological, economic, and recreational impacts (Simberloff et al. 2005).

Successes in invasive aquatic plant management depend upon knowledge of the biology and ecology of the target species (Nichols 1991, Moody et al. 2008). For example, research on the growth patterns and environmental preferences of invasive aquatic plants has informed the development of effective management strategies (Getsinger et al. 2008). In addition, clear understanding of the ecological and other impacts of aquatic invasive plants is vital for prioritizing management (Kumschick et al. 2012, Blackburn et al. 2014). Unfortunately, this foundational knowledge has not been

developed for all aquatic invasive plants. Among the invasive macrophyte species in the Great Lakes region, applied research needs are perhaps the most urgent for starry stonewort (*Nitellopsis obtusa*, Characeae; hereafter *Nitellopsis*) (Escobar et al. 2018b, Larkin et al. 2018).

Nitellopsis obtusa

Nitellopsis is a freshwater macroalga that is poorly understood in its invaded range of North America, despite its emergence as a problematic invader in the Great Lakes region. *Nitellopsis* is considered threatened across most of its native range in Europe and Asia but has expanded rapidly in its invaded range (Larkin et al. 2018). *Nitellopsis* was first recorded in North America in the St. Lawrence River in 1974 (Karol and Sleith 2017), but has become recognized as a widely established, high-priority invasive species in just the last decade. In the 1970s and early 1980s, *Nitellopsis* was found in Quebec, New York, and Michigan (Schloesser et al. 1986). It was roughly 20 years before any further invasions of *Nitellopsis* were recorded, but after that reports grew rapidly. This apparent rapid population growth following a multi-decade lag phase is consistent with other plant invasions, and suggests *Nitellopsis* is still expanding its invaded range in North America (Sleith et al. 2015, Escobar et al. 2016, Larkin et al. 2018). Today, *Nitellopsis* is present in two Canadian provinces and eight northern-tier states spanning from the Northeast to Midwest (Kipp et al. 2021). Most new infestations of *Nitellopsis* are discovered near public accesses, suggesting human-assisted movement between water bodies is a primary spread vector.

Minnesota is on the leading edge of *Nitellopsis* invasion in North America. The first recorded occurrence of *Nitellopsis* in Minnesota was from Lake Koronis (Stearns Co.) in 2015, and additional infestations have been discovered each year since. *Nitellopsis* infestations in Minnesota range from lake-wide nuisance levels (e.g., Lake Koronis), to small, localized patches (e.g., Grand Lake [Stearns Co.], Wolf Lake [Hubbard/Beltrami Co.]). The differences in invasion severity could be due to environmental characteristics of the invaded lake and/or an artifact of differences in time since invasion, which are unknown. For example, *Nitellopsis* could have been present in Lake Koronis well before it was discovered in 2015, which may explain its lake-wide prevalence. After the first discovery of *Nitellopsis* in the state, new surveillance efforts identified apparent early invasions wherein *Nitellopsis* was still restricted to isolated patches. Knowledge of conditions that may promote nuisance growth of *Nitellopsis* is currently lacking, but this information has been valuable for the management of other aquatic invasive plants (Royle et al. 2017). *Nitellopsis* infestations are aggressively managed in MN and there are limited untreated reference lakes to track natural growth and invasion patterns. But there is much to learn about the baseline biology and ecology of *Nitellopsis* from these unmanaged populations. Until 2018, *Nitellopsis* was only present in greater Minnesota, where it was clustered in the central and northern parts of the state (South: Stearns/Wright Counties, North: Beltrami/Cass Counties). However, the first occurrence of *Nitellopsis* in the Twin Cities Metropolitan Area was found in August of 2018 (Medicine Lake, Hennepin Co.) (EDDMapS 2020). This Metro infestation further elevated the perceived statewide threat of *Nitellopsis* spread because of high rates

of boater movement in that region (Kao et al. 2020). *Nitellopsis* is currently known to be present in 15 lakes in 6 counties across the state (EDDMapS 2020).

Ecological niche and habitat suitability modeling indicate that further expansion of *Nitellopsis* is likely, both into new regions of North America and into additional lakes within already invaded states like Minnesota and Wisconsin (Escobar et al. 2016, Romero-Alvarez et al. 2017, Muthukrishnan et al. 2018b). Management of well-established populations of *Nitellopsis* has proven difficult and long-term control elusive (Glisson et al. 2018). Further, control of *Nitellopsis* has potential non-target impacts since it is typically managed with copper-based algaecides, which are long-lasting in aquatic systems and can have negative impacts on fish and other aquatic animals (Helfrich et al. 2009). Making informed management decisions is difficult as the ecological impacts of *Nitellopsis* are not well understood, and there remain substantial knowledge gaps regarding its biology and ecology that need to be addressed to support informed response efforts.

Overview of study

I investigated two aspects of *Nitellopsis* ecology in this study – ecological impacts and environmental preferences – to support prioritization and management of this species. My research was motivated by a need to define the threat of *Nitellopsis* to lake ecosystems (impacts) and assess how broadly those impacts may occur within infested lakes (fine-scale environmental preferences). Specifically, I evaluated the effects of *Nitellopsis* on native macrophytes (aquatic vascular plants and macroalgae) using two different methodologies – space-for-time-substitution (2018) and time sequence sampling

(2017-2020). I assessed impacts to native macrophytes with respect to their abundance, diversity, species composition, and functional composition for a richer understanding of *Nitellopsis*' ecological role as an invader. Expecting that impacts of *Nitellopsis* would scale with its abundance, I searched for relationships between environmental conditions and *Nitellopsis* abundance with the goal of developing baseline knowledge for predicting where nuisance growth would be most likely. Chapter 2 addresses the vegetation field sampling and impacts assessment for *Nitellopsis*. Chapter 3 addresses the environmental field sampling and species-environment relationships.

This study contributes to the body of knowledge about *Nitellopsis*, an invasive species of growing concern in the Great Lakes region. Results from this work reduce uncertainty on the threat of *Nitellopsis*, which will inform prioritization and management of this species in Minnesota and across its invaded range. This study fills critical knowledge gaps on *Nitellopsis* ecology and highlights further research needs to address threats associated with this invader. This study also represents a robust evaluation of an invader's impacts using multiple lines of evidence, i.e., space-for-time-substitution and time sequence sampling. Invasion impact studies of this scope are limited in aquatic systems, and this work can be used as a model for future AIS impacts assessments. In particular, the four-year time sequence sampling highlighted the dynamic nature of this species' abundance and impacts, which supports common criticisms of impact studies that only measure impacts at a single point in time. Chapter 4 contains a summary of contributions from this study and provides guidance for managers responding to and attempting to control *Nitellopsis* across the landscape.

Chapter 2

Plant community impacts associated with *Nitellopsis obtusa* invasion

Summary

Knowledge of the ecological impacts of aquatic invasive species should be used to guide efforts to protect ecosystems and prioritize and manage non-native species invasions. However, aquatic invasive species are a large-scale, ever-increasing natural resource issue, and information on some emerging invaders is insufficient to direct response efforts. In this study, I evaluated plant community impacts of the invasive macroalga *Nitellopsis obtusa* (starry stonewort) to provide a better sense of the threat it poses to inland lake communities. I employed two different observational approaches in this study, space-for-time substitution and time sequence sampling, to investigate relationships between *Nitellopsis* abundance and the following native plant responses: (i) native macrophyte richness and abundance, (ii) abundance of individual species, (iii) abundance of functional groups, and (iv) species and functional group composition. Findings from both methodological approaches indicate that *Nitellopsis* has a high capacity to negatively affect native macrophytes and alter community and functional composition, and plant community impacts increased with abundance of *Nitellopsis*. However, time sequence sampling revealed that temporal variation in *Nitellopsis* abundance, which led to periods of relatively low abundance, may provide opportunities for native plant recovery following invasion. Overall, this study suggests *Nitellopsis* should be strongly prioritized given its potential for rapid, aggressive growth and capacity to damage native plant communities.

Introduction

Invasive species are a global threat to biodiversity and are pervasive drivers of ecological change (Simberloff et al. 2013). Biological invasions in lakes can be particularly harmful because lakes contain high concentrations of biodiversity and their ecological communities are easily disturbed due to the strong trophic links between aquatic organisms (Abell 2002, Shea and Chesson 2002, Gallardo et al. 2016). Aquatic invasive species are a significant conservation threat and, as a result, invasion studies in freshwater systems have primarily focused on effects on recipient biological communities and ecosystems (MacIsaac et al. 2011). Impact assessments for nonnative species are critical to guide response and management, as responses to invasive species should ideally be prioritized based on their known impacts (Blackburn et al. 2014). However, quantifying invasion impacts can be challenging because experiments are difficult to perform in aquatic habitats (Ricciardi and MacIsaac 2011), and invasive species can be either "drivers" or "passengers" of ecological change, making it difficult to determine causal effects (Macdougall and Turkington 2005).

Does an invasive species drive ecological change or simply take advantage of underlying environmental disturbances? This is a central question in invasion biology (Powell et al. 2011, Bauer 2012, Waller et al. 2016). Invasive species can be the cause (driver) of declines in native species and alteration of ecosystems through direct competition (Macdougall and Turkington 2005). However, invasive species can also be passengers of ecological change, as when anthropogenic stressors harm native species while having minimal or even beneficial effects on invasive species (Zedler and Kercher

2004). Invasive species that do act as drivers of change pose a more direct conservation threat, which can potentially be mitigated through management (Hermoso et al. 2011). Conversely, attempting to control an invasive species that is benefiting from covarying disturbance may be only treating a symptom of the problem, yielding little ecological benefit (Hobbs and Humphries 1995). Determining whether an invasive species is responsible for ecosystem change is critical because it has implications for conservation and management decisions. Study designs influence the ability to distinguish between these driver vs. passenger dynamics of invasions, and are an important consideration in impact studies (Forrest and Taylor 2002).

In observational studies of invasion impacts, there are two primary lines of evidence – space-for-time-substitution (SFTS) and pre/post invasion or time-sequence sampling (TSS). In SFTS designs, spatially separated sites representing a gradient of interest, e.g., invasion stage, are used to infer impacts. Comparing uninvaded and invaded plots is a classic study design, although recent work has shifted to assessing impacts across abundance gradients to better represent invader-native relationships (Hejda et al. 2009, Hulme et al. 2013) and because elucidating abundance-impact curves helps in setting management goals (Yokomizo et al. 2009). A problem with SFTS is that it does not provide direct evidence of native species being displaced by an invader and cannot distinguish between passenger or driver effects (Rosaen, Grover, and Spencer 2012). For this reason, conclusions from SFTS are often referred to as 'apparent impacts' (Pearson et al. 2016). In other words, observed differences across space may be due to additional co-varying factors, like disturbances or environmental changes, rather than the invasion

itself. For example, an invader may have colonized a habitat that was vacant or had low diversity, the absence of pre-invasion data in an SFTS study could lead to the false inference that the invasion *caused*, rather than exploited, the lack of native diversity (Muthukrishnan et al. 2018a). Nonetheless, SFTS can provide good approximation of invasion impacts and can be relatively easily and broadly applied, which is advantageous for rapidly assessing the threats posed by emerging invasions (Pickett 1989, Meiners and Cadenasso 2005, Thomaz et al. 2012).

Observing an invasion play out over time through TSS is less common, but this method allows for stronger inferences regarding possible invader displacement of native species (D'Antonio and Flory 2017). Additionally, invader abundance and impacts can change over time, and native species have been shown to recover following initial invasion (Strayer et al. 2006). Since TSS captures an invasion as it progresses, it also enables quantification of spread rate or other temporal processes, advancing understanding and prediction of the threat posed by the invader. Much attention has been given to survey design and analyses in invasion impact studies (Forrest and Taylor 2002, Simberloff et al. 2013, Jeschke et al. 2014). Integrating multiple lines of evidence and evaluating diverse responses is critical to obtaining accurate, unbiased assessments of invasion impacts (Thomaz et al. 2012).

Nonnative macrophytes are a major concern in freshwater ecosystems due to their capacity for ecological impacts. The tendency for macrophytes to become invasive is attributed to their broad environmental tolerances, high productivity, and high dispersal capacity (Chambers et al. 2008). Native macrophyte communities can be strongly

impacted by nonnative macrophyte invasions (Gallardo et al. 2016, Muthukrishnan et al. 2018a). This is of conservation concern because native macrophytes are critical ecosystem components that provide habitat complexity, regulate ecosystem processes, and support diversity across multiple trophic levels (Thomaz and Cunha 2010). The ecologically important role of native macrophytes is shaped by their functional characteristics, abundance, diversity, and composition – all of which invasive macrophytes can affect. Declines in species richness and abundance of native macrophytes are the most widely studied and reported impacts of invasive macrophytes (Stiers et al. 2011), and most of this evidence comes from correlative SFTS studies.

Using species richness alone to measure diversity effects can be problematic as it does not account for important changes to community composition associated with invasion (e.g., Sodhi *et al.*, 2019). For example, a potential effect of invasions is for rare native species to become rarer while generalist and non-native species expand, a process that can lead to biotic homogenization – ecological communities becoming more similar to each other over time – even without reductions in species richness (Olden 2006). This was recently illustrated in a study of Minnesota shallow lakes, which showed relatively rapid biotic homogenization that was exacerbated by the presence of invasive macrophytes, despite there being no significant effects of invasion on lake-level richness (Muthukrishnan and Larkin 2020). Species richness is a key measure of diversity, but effects on individual species, functional groups, and overall composition must also be considered to fully understand invasion impacts.

Species do not respond uniformly to invasion (Byun et al. 2018). Relationships between invasive macrophytes and individual native species can range from negative to neutral to even positive (Olson and Doherty 2014). Teasing out these relationships is important for conservation planning and habitat management, as with species that are of conservation concern or perform key ecological functions, e.g., providing waterfowl forage or suitable structure for fish spawning (Schallenberg et al. 2013). However, species-specific analyses can be difficult. Data for individual macrophyte species is often inadequate due to many native macrophytes being naturally rare and thus poorly represented in observational datasets (Hansen et al. 2013, Verhoeven et al. 2020). Extensive sampling is needed to collect sufficient data to draw conclusions on associations between individual species and an invader. However, groups of native plants that share similar traits, e.g., functional groups, are regulated by broad environmental gradients and are more consistently distributed (Schneider et al. 2018). Grouping functionally similar species can address problems with data gaps and illustrate more general ecological patterns. Functional-based analyses allow for robust testing of invasion impacts and strengthen inferences regarding invader-native relationships, given that outcomes of plant interactions are heavily influenced by species' respective traits (Magee et al. 2010, Kraft et al. 2014, Sodhi et al. 2019). For example, examining the effects of the canopy-forming invader *Myriophyllum spicatum* (Eurasian watermilfoil) on different life forms, Stiers et al. (2011) showed submerged and floating species declined while emergent species were unaffected, suggesting direct competition for space or light could be the mechanisms driving impacts.

Community composition may also provide signals of invasion impacts that are not reflected in broad diversity measures alone (e.g., richness, abundance). In general, invasion tends to make communities more similar over time, i.e., biotic homogenization (Castro-Díez et al. 2016). Compositional changes should not be overlooked in macrophyte invasions as plant community composition influences habitat structure (Thomaz and Cunha 2010), changes to which can have negative impacts on other trophic levels, such as benthic invertebrates and fishes (Gallardo et al. 2016). Species composition is sensitive to change following macrophyte invasion (Thomaz and Cunha 2010, Stiers et al. 2011). Additionally, the functional composition of communities can also be strongly affected (Michelan et al. 2010).

Regardless of the native responses evaluated in invasion impact studies, most evidence comes from SFTS studies, and TSS studies applied to invasive macrophytes are particularly rare. However, those studies that have been performed provide insights by quantifying native species displacement, compositional changes, and invader expansion rates over time. Biotic homogenization (Muthukrishnan and Larkin 2020) and declines in species richness (Muthukrishnan et al. 2018a) associated with invasion were confirmed through temporal analyses of repeatedly sampled lakes in Minnesota. A TSS study in a New York lake showed a rapid increase in *Myriophyllum spicatum*, from 15% to >95% frequency of occurrence in only a three-year period, while average native richness declined from 5.5 to ~1 species per sampling point (Boylen et al. 1999). These findings highlight the extent to which TSS approaches can more fully elucidate the impacts of invasive macrophytes.

Invasive plants pose significant threats to aquatic systems, but impacts are complex and dependent upon the characteristics of individual invaders (Stohlgren and Rejmánek 2014). Most research on invasive macrophytes has focused on a few high-profile species with long invasion histories, such as *Eichhornia crassipes* (water hyacinth) and *Myriophyllum spicatum*. In contrast, information on emerging nonnative macrophytes is limited and there is uncertainty regarding the ecological threats they pose. This is especially true for *Nitellopsis obtusa* (starry stonewort; hereafter *Nitellopsis*), an invasive characean macroalga with a growing presence in the Great Lakes region. *Nitellopsis* was first identified in North America in 1974 (Karol et al. 2017), but received little attention until ~2012, after which reporting of new localities rapidly increased (Larkin et al. 2018). The invaded range of *Nitellopsis* now includes two Canadian provinces and eight northern-tier states spanning from Vermont in the northeast to Minnesota in the Midwest (Kipp et al. 2021).

Nitellopsis is the only invasive freshwater macroalga in the region. Most invasive macroalga are marine species, and invasive vascular macrophytes and macroalga differ significantly in their biology and ecology. Therefore, there is little background literature to draw upon, and current information on *Nitellopsis* ecology is insufficient to predict the threat it poses to lake plant communities. Two papers have described effects of *Nitellopsis* on native macrophytes, only one of which drew conclusions based on systematically collected data (Pullman and Crawford 2010, Brainard and Schulz 2016); both suggest *Nitellopsis* should be categorized as a high priority invader. Pullman and Crawford (2010) anecdotally described *Nitellopsis* as having significant negative

ecological effects in Michigan lakes. Brainard and Schulz (2016) documented declines in native macrophyte biomass and richness correlated with increasing *Nitellopsis* in four New York lakes. This study was the first and only rigorous evidence to date that *Nitellopsis* negatively affected native macrophytes (Brainard and Schulz 2016). However, Brainard and Schulz (2016) used SFTS and noted uncertainties in whether *Nitellopsis* displaces native macrophytes or simply capitalizes on disturbed/poor quality habitat that was previously underutilized. TSS data are needed to assess potential native displacement by *Nitellopsis* and community changes following invasion. There is also no published information on the effects of *Nitellopsis* on individual species, functional groups, or overall community composition. These represent critical knowledge gaps regarding the impacts of *Nitellopsis*. In sum, current data on the impacts of *Nitellopsis* is limited in extent, geographic range, and scope.

The goal of my work was to comprehensively evaluate the effects of *Nitellopsis* on native macrophytes. This study complements previous work and establishes entirely new findings on the ecological impacts of *Nitellopsi*s. I collected both SFTS and TSS data to evaluate impacts of *Nitellopsis*. For part one of this study, I used an SFTS approach to examine relationships between *Nitellopsis* abundance and the following native responses: (i) native macrophyte richness and abundance, (ii) abundance of individual species, (iii) abundance of functional groups, and (iv) species and functional group composition. I hypothesized that *Nitellopsis* would be associated with reductions in native macrophytes and changes in the composition of macrophyte assemblages. For the second part of this study, I used a TSS approach to monitor *Nitellopsis* invasion and

native macrophytes over time. I hypothesized that *Nitellopsis* infestations would increase in extent (presence) and severity (cover), which would lead to displacement and declines in native macrophytes. I expected impacts identified from TSS data would be consistent with observations from SFTS data. This study advances the body of knowledge on *Nitellopsis* by addressing impacts in a new geographic region, quantifying previously unmeasured impacts, and providing the strongest evidence to date of rapid local spread and displacement of native species. This work also provides a foundation for future research on impacts to other trophic levels or ecosystem processes.

Methods

Study lakes

Data were collected from 2017-2020 in the months of July and August, coinciding with peak biomass for many native macrophytes and a period of rapid biomass accumulation for *Nitellopsis*. Three Minnesota lakes invaded by *Nitellopsis* were sampled; SFTS sampling was employed in all lakes and TSS in two lakes. There are currently 14 lakes in the state with known *Nitellopsis* populations. Of the 14, 3 were most suitable for use as study lakes: Koronis (Stearns Co.), Moose (Beltrami Co.), and Winnibigoshish (Cass/Itasca Co) (Figure 2.1). These lakes all contained sufficient areas of unmanaged *Nitellopsis* to enable extensive sampling. In the other infested lakes in Minnesota, *Nitellopsis* is restricted to small, localized areas and/or subjected to control efforts across its distribution. A priority was to maximize the generality of results by examining *Nitellopsis* invasion in multiple lakes and regions of Minnesota, thereby

encompassing differences in environmental conditions and plant communities. The selected study lakes supported these objectives.

Koronis, Moose, and Winnibigoshish are representative of the diverse types of lakes *Nitellopsis* can invade (Muthukrishnan et al. 2018b). Koronis is located in central Minnesota and, in 2015, was the first lake in the state found to have *Nitellopsis*. It is a 1,197 surface-ha drainage lake classified as slightly eutrophic (mean Trophic State Index = 54; total phosphorus = 47 µg/L), with a maximum depth of 40.2 m. Koronis is the only study lake where large-scale management (chemical and mechanical treatments) occurred close to the sampling period. All sampling occurred >200 m away from control efforts. Moose and Winnibigoshish are located in north-central Minnesota and *Nitellopsis* was first identified in both in 2016. Moose is a 243-ha seepage lake classified as mesotrophic (mean TSI = 44; total P = 18 µg/L), with a maximum depth of 21.6 m. Winnibigoshish is a 21,620-ha drainage lake that is a flow-through for the Mississippi River; it is classified as mesotrophic (mean TSI = 47, total P = 20.7 µg/L), with a maximum depth of 21.3 m.

Correlations between native macrophytes and Nitellopsis obtusa *(SFTS approach)*

Transects along invasion gradients (SFTS data) were used to document associations between *Nitellopsis* and various attributes of native macrophyte assemblages. Sampling for this objective was performed in July and August of 2018 (Table 2.1). Extensive sampling occurred within the known extent of *Nitellopsis* in Koronis, Moose, and Winnibigoshish (Figure 2.1). Salection of transect locations were informed by point-intercept surveys conducted by the Minnesota Department of Natural Resources (MN DNR) to delineate the extent of *Nitellopsis* within these lakes. Transects

were positioned to capture transitions from *Nitellopsis*-dominated to native-dominated areas; one end of each transect had dominant *Nitellopsis* (90-100 percent cover) while the other end had no or minimal *Nitellopsis* (0-10 percent cover). This approach was used to capture abundance-impact relationships that can be informative for setting management goals (Yokomizo et al. 2009), and because only comparing uninvaded vs. heavily invaded plots can introduce error and bias impact assessments (Hulme et al. 2013). Transects were 20-m long and spaced a minimum of 75-m apart within the littoral zone. Transects were placed without regard to depth or orientation to shore. Depth was measured at the ends and midpoint of transects, its influence was tested for post-hoc. A total of 68 transects (Koronis = 26, Moose = 25, Winnibigoshish = 17) were sampled, comprising 1,365 observations (quadrats). Winnibigoshish had fewer transects because *Nitellopsis* grew sparsely there and was difficult to locate, even though it was distributed across a large area. This contrasted with the matted, dense growth observed in Moose and Koronis and may be due to the coarse, sandy sediment observed in Winnibigoshish or differences in invasion history.

Along transects, vegetation data were collected using a belt-transect method with quadrat sampling units (Parker et al. 2011, Madsen and Wersal 2017). Divers swam along transects and sampled consecutive 1-m^2 PVC quadrats, centered on the transect tape (20-m transects comprising 19 quadrats each). In each quadrat, total native macrophyte cover and individual species cover were estimated using an arcsine-square root cover class system (0=absent, 1=0-1%, 2=1-5%, 3=5-25%, 4=25-50%, 5=50-75%, 6=75-95%, 7=95-99%, 8=99-100%) (McCune et al. 2002).

All macrophytes were identified to species with the exception of *Chara* (Muskgrass) species, which were recorded as *Chara* spp. due to cryptic species differences. In all, four observers performed sampling, but each sampling unit (transect) had a single observer. Visual estimation of plant cover can be inconsistent between observers (Madsen and Wersal 2017). To minimize observer bias, all observers attended a macrophyte identification workshop and participated in a group training day prior to sampling.

To characterize different functional types of native plants, which may represent different competitive abilities (Goldberg and Barton 1992) and thus susceptibilities to *Nitellopsis* invasion, macrophytes were separated into seven groups based on life form/morphology (Figure 2.2). The specificity of grouping is similar to Schneider *et al.* (2018). Native macrophyte groups were classified based on the following hierarchy of categorical traits: rooting (yes/no), height (short/tall/caulescent), leaf-type (floating/submerged), stature (upright, carpet-forming, semi-spreading), and leaf size (broad/narrow). These traits were selected based on expectations that they may influence the ability of native macrophytes to co-occur, or not, with *Nitellopsis*. For example, unrooted species might be less affected by *Nitellopsis* because they do not have to attach to sediments beneath dense stands of *Nitellopsis*. In contrast, species that are functionally similar to *Nitellopsis* (e.g., carpet-forming) may have greater niche overlap and vulnerability to competitive displacement (Price et al. 2013). This grouping approach resulted in seven distinct groups: 1) unrooted, short statured; 2) rooted, basal; 3) rooted, caulescent, floating leaves; 4) rooted, caulescent, submersed, semi-spreading, narrow-

leaved; 5) rooted, caulescent, submersed, semi-spreading, broad-leaved; 6) rooted, caulescent, submersed, carpet-forming; and 7) rooted, caulescent, submersed, upright (Figure 2.2). These groups were used to create derived variables for functional group abundance based on species occurrences recorded in quadrats (i.e., the cover of species representing each group were summed per quadrat to create aggregate abundance values for each group). This enabled analyses to be performed on both individual functional groups and overall functional composition.

Native community changes associated with Nitellopsis obtusa *invasion over time (TSS approach)*

Resampling of permanently established transects ('TSS transects') was used to generate TSS data to enable more robust inferences regarding *Nitellopsis'* effects on native species, diversity, and community composition. In 2017, TSS transects were established and sampled in Koronis and Moose (TSS transects were not established in Winnibigoshish). Transects were set up along invasion gradients as described for the SFTS approach, but these were permanently marked with metal stakes so that exact localities could be repeatedly sampled. In Koronis and Moose, there were five sites, each with three parallel, 30-m transects spaced ~2-m apart (15 total transects per lake). Along these transects, 1-m^2 quadrats were sampled at 2-m intervals (every other meter). All species present were recorded and their cover was estimated using the arc-sine square root cover class system described above. Total native cover was not recorded during the first year of TSS sampling, and thus is not included in analysis of temporal trends.

In 2017, vegetation data were collected from the 30 permanent transects. In 2018, SFTS transect sampling was prioritized and only four transects in Koronis were resampled. In 2019, an effort was made to resample all permanent transects in Koronis and Moose. All transects in Koronis were resampled; however, only 9 of 15 transects were resampled in Moose—the other 6 could not be relocated because of extreme *Nitellopsis* height and density. In 2020, all permanent transects in both Moose and Koronis were resampled; this included the transects in Moose that could not be relocated in 2019, likely because *Nitellopsis* density had declined. In sum, the TSS dataset spans four years total and the dataset is unbalanced as not all transects were revisited annually (see Table 2.1 for TSS transect sampling history). TSS data were used to analyze change over time in *Nitellopsis* abundance and native macrophyte assemblages.

Statistical Analyses

R statistical software version 3.4.1 was used to perform all analyses for this study (R Core Team 2017). Data analysis for SFTS is described first, followed by methods for the TSS data. The following responses were evaluated for both datasets: native macrophyte richness, species composition, functional composition, and abundance of individual species and functional groups. Total native cover was recorded and analyzed for SFTS but not TSS. *Nitellopsis* cover was evaluated as a response for TSS data. Generalized linear mixed effects models (GLMMs) that allow for discrete (integer) response variables (i.e., cover classes, species richness) and non-independent data (multiple quadrats sampled within single transects and quadrats repeatedly sampled at multiple time points) were used. A multivariate extension of generalized linear models

was also used to evaluate differences in community composition associated with varying *Nitellopsis* abundance. Univariate GLMs were used to evaluate effects of *Nitellopsis* on individual species and functional groups.

GLMMs with a Poisson error distribution were used to evaluate the relationship between *Nitellopsis* and the responses species richness and total native cover on SFTS transects. All GLMMs were fit using the lme4 package (Bates et al. 2015). Fixed effects for the models were *Nitellopsis* cover and Lake ID. The models included transect ID as a random effect. Equation 1 shows the model for native richness; the distribution and predictor variables were the same for the total native cover model.

$$Richness_{i,j} = species\ richness\ in\ the\ i^{th}\ quadrat\ from\ the\ j^{th}\ transect$$

$$Richness_{i,j} \mid x_i \sim Poisson(\lambda_{i,j})$$

$$log\left(\lambda_{i,j}\right) = B_{o,j} + B_1\left(Nitellopsis_{i,j}\right) + B_2\left(Lake_j\right) - 1$$

GLMMs were evaluated for validity following Bolker et al. (2008). Significance of fixed effects was assessed by running parametric bootstrap comparisons (n = 500) between a model fit with and without each fixed effect (Halekoh and Hojsgaard 2014). Bootstrap sampling (n = 500) was used to obtain accurate confidence intervals around model parameters.

A multivariate generalized linear model (mvGLM) with a negative binomial error distribution was implemented in the mvabund package (Wang et al. 2019) to evaluate the relationship between *Nitellopsis* cover and species composition. MvGLMs are a GLM

analog with multivariate response variables that can be used to test for effects of predictors on community composition (Wang et al. 2012). For this model, the entire native species matrix was used as the response, with *Nitellopsis* cover as a predictor. Hierarchical data (quadrats nested within transects) were accounted for using restricted permutations during hypothesis testing. Specifically, significant effects of *Nitellopsis* on species composition were tested for using analysis of deviance with resampling within transects (n = 500). Univariate GLMs were then fit to evaluate species-specific responses to *Nitellopsis*, with the same error distribution and testing of *Nitellopsis* effects as described for mvGLM.

An mvGLM as described above was also used to evaluate the relationship between *Nitellopsis* and functional group composition. The functional group cover matrix was used as the response with *Nitellopsis* cover as a predictor. The significance of predictors was evaluated using the analysis of deviance resampling procedure described above. Univariate GLMs were then fit to evaluate the response of individual functional groups to *Nitellopsis* abundance.

TSS data were analyzed separately for Moose and Koronis because of inconsistencies in sampling and unbalanced data. All models described below were fit for both Koronis and Moose. GLMMs, as described for SFTS data, were also used for this dataset to account for non-independence of data associated with resampling of transects. GLMMs with a binomial error distribution (i.e., a logistic mixed effects model) were used to evaluate how *Nitellopsis* invasion progressed on transects over the study period. Presence or absence of *Nitellopsis* in the sampled quadrats was used as a binary response

variable, year was included as a categorical fixed effect, and transect ID was included as a random effect to account for repeated sampling of the same locations over time. The same model structure was also used to evaluate how the distribution of native macrophytes changed over the study period, with presence/absence of native species in quadrats as the response variable and year and transect ID again included as fixed and random effects, respectively.

A GLMM with a Poisson error distribution was used to evaluate changes in *Nitellopsis* cover over the study period. Year was included as a fixed effect and transect ID as a random effect to account for resampling over time. The model was effects-coded because the magnitude of change over time was the primary interest, i.e., the intercept represents the year-one state and the other model coefficients represent the difference between a given resampling year and year 1. A second Poisson GLMM was fit to evaluate how native richness changed over time and in response to *Nitellopsis* cover. Fixed effects included year and *Nitellopsis* cover, with transect ID as a random effect to account for resampling over time.

An mvGLM with a negative binomial error distribution was used to evaluate changes in species composition associated with *Nitellopsis* invasion of TSS transects. A matrix of native species data was used as the response. The predictors included were year and *Nitellopsis* cover. Non-independence of data associated with hierarchical sampling (multiple transects sampled within sites) and repeated measures (resampling of transects) was accounted for using restricted permutations during hypothesis testing. In each site, there were three TSS transects spaced ~2-m apart. Site was used as a blocking factor for

restricted permutations as vegetation within sites was relatively homogenous compared to between sites. Analysis of deviance was used to evaluate significance of predictors, and bootstraps (n = 500) were computed via resampling observations within individual sites. Univariate GLMs were then fit to evaluate how individual species changed over time and in response to *Nitellopsis*, and p-values were adjusted for multiple testing.

An mvGLM as described above was also used to evaluate changes in functional composition associated with *Nitellopsis* invasion of TSS transects. The functional group cover matrix was used as the response. Year and *Nitellopsis* were included as predictors, and data were permuted within sites. The significance of predictors was evaluated using the resampling procedure described above. Univariate GLMs were then fit to evaluate how individual functional groups changed over time and in response to *Nitellopsis*, and p-values were adjusted for multiple testing.

Results

Correlations between native macrophytes and Nitellopsis obtusa *(SFTS approach)*

Thirteen native macrophyte species were found in Koronis, 23 in Moose, and 18 in Winnibigoshish. In each lake, all native macrophytes identified co-occurred within a quadrat with *Nitellopsis* at least once, i.e., no species were found only in uninvaded quadrats. Native macrophytes commonly co-occurring with *Nitellopsis* (found in >75% of invaded quadrats) were *Chara* spp. in Moose and Winnibigoshish, *Najas guadalupensis* (southern naiad) in Moose, and *Najas flexilis* (slender naiad) in Winnibigoshish. Five out of eight morphological groups – carpet, dissected-rooted,

dissected-unrooted, broad-pondweeds, and narrow-pondweeds – were found in all three study lakes. Average *Nitellopsis* cover was 4.2 ± 2.7 (~30% cover) in Koronis, 4.1 ± 3.4 (~27% cover) in Moose, and 4.6 ± 2.3 (~40% cover) in Winnibigoshish. Average native cover was 4.4 ± 2.4 (~35% cover) in Koronis, 4.6 ± 2.9 (~40% cover) in Moose, and 3.3 ± 1.7 (~21% cover) in Winnibigoshish. Average native richness (per 1 m^2) was 2.2 ± 1.3 in Koronis, 3.4 ± 2.2 in Moose, and 3.8 ± 2.0 in Winnibigoshish.

Species richness declined with increasing *Nitellopsis* cover in all lakes (Figure 2.3). The decrease in species richness from quadrats with no *Nitellopsis* to quadrats with its maximum observed cover was approximately one species, a reduction of approximately 1 in 2 to 1 in 4 species on average. *Nitellopsis* cover was a significant predictor of species richness, and species richness differed significantly between lakes. See Table 2.2 for full results of all SFTS GLMM models.

Native macrophyte cover declined sharply with increasing *Nitellopsis* cover in all lakes (Figure 2.4). There was a roughly 1:1 negative relationship between native cover and *Nitellopsis* cover. In the absence of *Nitellopsis*, native cover averaged 95-99% in Winnibigoshish and 99-100% in Koronis and Moose. Under maximum *Nitellopsis* cover, native cover averaged just ~1-5% in all three study lakes. *Nitellopsis* was a significant predictor of cover and cover differed significantly between lakes. See Table 2.2 for full model results.

Nitellopsis influenced the diversity and composition of native macrophyte communities. Species composition significantly differed as a function of *Nitellopsis* abundance (mvGLM: $p = 0.001$), as did abundance of 5 out of the 31 native macrophytes

species found in this study (Table 2.3). *Nitellopsis* was also associated with altered functional composition of the macrophyte community (mvGLM: $p = 0.001$) and had a significant effect on seven out of the eight functional groups (Table 2.4).

Native community changes associated with Nitellopsis obtusa *invasion over time (TSS approach)*

Overall, *Nitellopsis* expanded and increased in abundance on Koronis TSS transects while presence of native vegetation decreased, despite increases in richness over the four-year study period. On Moose TSS transects, *Nitellopsis* presence and cover and native richness also increased over time; however, presence of native vegetation did not significantly change. These patterns are described in greater detail in the following paragraphs.

On both Koronis and Moose TSS transects, expansion of *Nitellopsis* was supported by significant, positive effects of year on the chances of quadrats being invaded (Table 2.5). In Koronis, quadrats were 39 times more likely to be invaded in year 4 compared to year 1. In Moose, quadrats were 8 times more likely to be invaded in year 4 compared to year 1. Native vegetation was significantly diminished in Koronis, indicated by a negative effect of year on the presence of any native vegetation. Quadrats were 24 times less likely to contain native vegetation in year 4 compared to 1. Presence of native vegetation did not change over the study period in Moose.

For both Koronis and Moose TSS transects, increases in *Nitellopsis* abundance over time were supported by significant positive effects of year on *Nitellopsis* cover

(Table 2.6). In Koronis, mean cover of *Nitellopsis* was 3.1 (~7% cover) in year 1 and had increased to 5.7 (~49%) in year 4; however, the highest mean cover was observed in year 3: 6.4 (~84%) (Figure 2.5). In Moose, mean cover of *Nitellopsis* was 1.9 (< 1% cover) in year 1 and had increased to 3.92 (~23%) in year 4 (Figure 2.5).

Species richness increased over the study period on both Koronis and Moose TSS transects (Table 2.6). However, richness steadily increased each year in Moose while, in Koronis, it initially declined before recovering the following year. Between years one and three in Koronis, native richness declined significantly from an average of 2.5 to 1.9 species; however, average richness in year 4 was significantly higher than in year 1 (Figure 2.6). Richness was lowest in year 3 when *Nitellopsis* cover peaked. *Nitellopsis* cover was negatively associated with native richness across years: for every unit increase in *Nitellopsis*, native richness declined by ~0.9 species; this is consistent with the magnitude of negative effects inferred from the SFTS data. Between years 1 and 4 on Moose, native richness increased significantly: from an average of 3.2 to 6.5 species (Figure 2.7). Despite overall increases in richness across years, *Nitellopsis* cover had a significant negative effect on species richness in Moose, with a similar effect size as found for Moose SFTS data and Koronis TSS and SFTS data (~1:1 negative ratio).

Composition of species and functional groups changed over time and as a function of *Nitellopsis* abundance on both Koronis and Moose TSS transects (mvGLM: $p = < 0.001$ for all four models). In Koronis, 4 out of 14 species showed a significant negative response to *Nitellopsis* cover: *Ceratophyllum demersum*, *Chara* spp., *Stuckenia pectinata*, and *Potamogeton foliosus*. With the exception of *Ceratophyllum demersum*,

cover of these species was diminished in year 4 compared to year 1. *Chara* spp., and *Stuckenia pectinata* were also identified as being negatively affected by *Nitellopsis* in SFTS data. In Moose, 10 out of 23 species showed a significant negative response to *Nitellopsis*: *Ceratophyllum demersum, Stuckenia pectinata,* as in Koronis, and additionally, *Myriophyllum sibiricum, Najas guadalupensis, Potamogeton amplifolius, Potamogeton friesii, Potamogeton illinoensis, Potamogeton pusillus, Potamogeton praelongus, Potamogeton zosteriformis*. Of these, only cover of *Najas guadalupensis* was diminished at the end of the study period compared to year 1. In addition to the species mentioned previously, negative effects of *Nitellopsis* cover on *Potamogeton illinoensis* was consistent with SFTS data. In Koronis, three out of seven functional groups showed a significant negative response to *Nitellopsis*: carpet-forming, dissected-unrooted, and narrow-leaved pondweeds. Cover of these groups was diminished at the end of the study period compared to year 1. In Moose, seven out of eight functional groups showed a significant negative response to *Nitellopsis*: carpet-forming, dissected-unrooted, and narrow-leaved pondweeds, as in Koronis, and additionally basal, dissected-rooted, and broad-leaved pondweeds. Of these, carpet-forming was the only group that had declined at the end of the study period. Negative effects of *Nitellopsis* on groups identified in the TSS data were consistent with results from the SFTS data.

Discussion

Over the last decade, *Nitellopsis* has emerged as a high-profile invader in the Great Lakes region, yet there has been minimal information on the ecological consequences of its invasion. Evidence presented here fills key knowledge gaps with

regard to *Nitellopsis'* potential plant community impacts, information that can support prioritization and response strategies for this invader. Overall, it appears that *Nitellopsis* has a high capacity to negatively affect native macrophytes and alter community and functional composition, lending support to past anecdotal claims of ecological impacts. *Nitellopsis* is capable of aggressive growth that can create extensive, nearly monotypic vegetation and displace native species. However, it is important to note that its abundance and impacts differed among lakes and between years, there is thus likely to be high variation in outcomes of invasion. Temporal variation in *Nitellopsis* abundance could allow recovery of native species during periods of lower *Nitellopsis* abundance. In addition to existing concerns about impacts to recreation and other human uses of invaded lakes, loss of plant diversity and alteration of habitat structure documented in this study should be considered in threat assessments for this species.

Correlations between native macrophytes and Nitellopsis obtusa *(SFTS approach)*

Impacts of invasive species can be context-dependent (Kumschick et al. 2015), and this should be considered when applying these results to other lakes infested with *Nitellopsis*. All evidence presented here came from unmanaged plant communities and impacts of *Nitellopsis* are likely to be mediated by control efforts—though the potential benefits of reduced *Nitellopsis* abundance need to be weighed against possible non-target effects on native species. Environmental conditions or features of the recipient plant communities are also likely to influence the outcome of *Nitellopsis* invasion and could lead to different outcomes than those observed here. However, the consistency of results across study lakes and sampling methods support the observed negative impacts being

applicable to other systems. Patterns were consistent across the three study lakes, despite their differences in plant communities and environmental conditions. All results are from Minnesota lakes, a relatively small geographic subset of *Nitellopsis*' invaded range; however, they are consistent with observations from New York state (Brainard and Schulz 2016) and the functional group analyses may be more generalizable to lakes with different macrophyte species pools. Future research on the impacts of *Nitellopsis* can build upon the findings of this study and be used to evaluate if the patterns observed here hold across geographic regions. This will be particularly important as the invaded range of *Nitellopsis* in North America continues to expand.

Potential drawbacks of this study were that site selection for transects was not randomized and environmental conditions (e.g., depth) were not systematically controlled for. However, environmental conditions were measured on SFTS transects (see Chapter 3 on environmental associations). Subjective selection of sampling locations was used to ensure that sampling would capture transitions in *Nitellopsis* abundance and the diversity of native macrophytes in each lake; random site selection would not have fulfilled these study criteria. However, results from non-subjective sampling designs (i.e., point intercept surveys) informed sampling locations, so sites were broadly distributed across the known extent of *Nitellopsis* in each lake. A potential concern with my method of transect placement is that transects could have aligned with environmental gradients that covaried with transitions in vegetation. However, habitat conditions appeared relatively homogenous with no obvious environmental gradients (see also Chapter 3). Some transects were placed on gradual slopes, but Brainard and Schulz (2016) showed that

relationships between *Nitellopsis* and native species were consistent across varying depths. Another concern is that differences in depth or other characteristics between transects could influence the vegetation present, but this was accounted for by the statistical methods used (and additionally, environment-species relationships on transects were explored in Chapter 3). Specifically, the influence of site-specific conditions was minimized through the use of mixed effects models, with each transect having its own intercept to account for underlying variation in environmental conditions or macrophyte communities.

The use of SFTS in impact studies is often critiqued because there is no way to discern if the invader is a causal driver or covarying passenger of observed trends. This uncertainty was dealt with in this study by pairing SFTS and TSS approaches, enabling multiple lines of evidence to be considered. The observed associations between *Nitellopsis* and native macrophytes in the SFTS data likely reflect actual impacts given their consistency with TSS patterns. Additionally, native cover was consistently high in the absence of *Nitellopsis*, suggesting that *Nitellopsis* was not simply colonizing otherwise underutilized habitat. Factors other than *Nitellopsis* invasion were minimized in this study as all sampling occurred in unmanaged areas with no other invasive species present. *Nitellopsis* may additionally benefit from disturbance and these processes could interact; however, the purpose of this study was to establish baseline knowledge of *Nitellopsis*' impacts and synergistic effects were not investigated. Overall, my findings suggest that *Nitellopsis* is a direct driver of community change and that SFTS evidence was representative of its impacts.

Native macrophytes were increasingly affected as *Nitellopsis* increased, consistent with ecological impacts generally scaling with invader dominance (Hejda et al. 2009). Management goals should focus on minimizing *Nitellopsis* abundance, as evidenced by the linear cover-impact relationship documented in this study. Most often, *Nitellopsis* was either absent or present at nearly 100% cover during this study. This suggests *Nitellopsis* is an aggressive invader with a high capacity to become the dominant species where it establishes. The rapid and abundant growth of invasive plants leads to increased competition that can drive declines in native species (Stiers et al. 2011). The observed declines in native richness and abundance associated with *Nitellopsis* is consistent with the findings of Brainard and Schulz (2016) from New York State, although effect sizes are not directly comparable due to methodological differences. *Nitellopsis* clearly has a strong negative relationship with native macrophytes at fine spatial scales (1-m^2 quadrats). Given that *Nitellopsis* is capable of becoming a lake-wide problem (e.g., >50% frequency of occurrence in the littoral zone of Koronis), these local declines in native species could result in significant lake-wide changes. Lake-wide declines in the richness and abundance of native plant species with *Nitellopsis* invasion have been suggested by point-intercept surveys of other infested lakes (Harman and Albright 2012, Russell and Genco 2014, Jurek and Hauck Jacobs 2020).

Changes associated with *Nitellopsis* cover significantly altered macrophyte assemblages based on community composition analyses. The data suggest this was a result of changes to both presence and abundance of natives. Changes to composition have been associated with other macrophyte invasions (Michelan et al.

2010, Stiers et al. 2011, De Amorim et al. 2015) and with macroalgal invasions in marine systems (Hewitt and Schaffelke 2007). Changes to community composition affect the physical structure of habitat and can have cascading effects on other organisms in aquatic systems (Thomaz and Cunha 2010). The ability of *Nitellopsis* to alter habitat structure underscores the need to investigate potential impacts to macroinvertebrates, fish, and other key organisms.

Species-level impacts of *Nitellopsis* were somewhat inconsistent and effects were only detected for a few common species. There was likely insufficient statistical power to detect effects of *Nitellopsis* on native species with limited occurrences, thus, a lack of significance should not be interpreted as evidence for the lack of biological effect. Indeed, *Nitellopsis* altered overall species composition, indicating it had a comprehensive effect on the presence and abundance of multiple species observed in this study. Impacts of *Nitellopsis* were detected for *Stuckenia pectinata*, which was a commonly lost species on Koronis TSS transects as *Nitellopsis* increased. Strong negative effects were also observed on two native dominants, *Chara* spp. and *Najas guadalupensis.* The ability to reduce native dominants is an indicator of highly competitive invaders (Vila and Weiner 2004) that can have significant community-level effects (Hulme et al. 2013).

Nitellopsis appeared to influence a broad range of native macrophytes based on functional group analyses. These widespread effects contrasted with the greater variability of outcomes observed in species-specific analyses, likely because pooling species by functional groups led to higher power to detect effects. All groups, except for

floating-leaved species, were negatively affected by *Nitellopsis* to some extent. This suggests that competition for space and/or light may be driving impacts of *Nitellopsis*. Species with a floating-leaf growth form are able to escape competition with *Nitellopsis* for space in the water column and can overtop *Nitellopsis* for light acquisition, advantages that other groups lacked. The lack of observed impacts to floating-leaved species supports field observations by Pullman and Crawford (2010) of water lily stands persisting above dense mats of *Nitellopsis*. The carpet-forming group, which shares a similar growth form with *Nitellopsis*, showed the strongest negative response to *Nitellopsis*. This suggests that *Nitellopsis* is capable of invading habitat where its niche is already occupied by similar species. *Nitellopsis* produces much more biomass than native species with similar growth strategies (Larkin et al., unpub. data, 2016-2019), which may allow it to outcompete established stands of vegetation. Based on the group-level effects identified in this study, it is likely that *Nitellopsis* invasion homogenizes the physical structure of macrophyte communities, which may have negative impacts on habitat quality for aquatic animals or other groups.

There are many potential mechanisms that could explain the negative effects of *Nitellopsis*, but these can only be partially addressed due to the observational nature of this study. *Nitellopsis* forms dense mats that leave little interstitial space for growth of other plants; this growth may function like a benthic barrier—monopolizing space, depleting light, and altering nutrient dynamics. Competitive displacement is indicated by the strong negative effects of *Nitellopsis* on species/groups that occupy the same space. Additionally, invaders with the greatest

community-level impacts tend to have greater cover and height relative to native dominants (Hulme et al. 2013). *Nitellopsis* is taller than native charophytes (Larkin et al. 2018) and *Najas* spp. and also had higher average cover than other native taxa in this study. The high abundance potential of *Nitellopsis* relative to native species is also consistent with biomass sampling performed on Moose and Koronis as part of a phenology study (Larkin et al., unpub. data, 2016-2019). Dense mats of *Nitellopsis* may also diminish light availability for species that do not grow to the surface, which could impede growth of native macrophytes that sprout from seed or are short-statured.

The 'unlike invader' hypothesis could also explain the strong negative effects of *Nitellopsis* observed in this study (Strauss et al. 2006). *Nitellopsis* is ecologically and morphologically distinct from vascular macrophytes, which comprise a majority of submersed aquatic vegetation in freshwater systems. Additionally, *Nitellopsis* is the only species in its genus, making it relatively distinct from other native charophytes in its invaded range. Introduced species that are less related to the resident plant community tend to have more severe impacts (Ricciardi and Atkinson 2004); but see (Fleming et al. 2014). While this study did not establish the mechanisms responsible for *Nitellopsis*' effects on native plants, the resulting patterns are nonetheless informative for guiding conservation and management responses.

Native community changes associated with Nitellopsis obtusa *invasion over time (TSS approach)*

Multi-year invasion time series in aquatic systems are uncommon, but this work demonstrates their utility for understanding invader impacts. This dataset reinforced findings from SFTS work, chiefly that impacts increase with *Nitellopsis* abundance and its invasion has direct effects on the diversity, abundance, and composition of native macrophytes. However, the temporal aspect of these data also provided context on the impacts of *Nitellopsis* that would not have been apparent from a single point in time. Specifically, expansion and abundance of *Nitellopsis* showed large interannual variation rather than steady increases over the time frame of the study. Periods of low *Nitellopsis* abundance may provide opportunities for native species to recover. However, it remains to be seen whether this interannual variability holds over longer time periods or simply adds variation to a longer-term trajectory of increasing *Nitellopsis*.

It is important to consider the caveats of this study when interpreting TSS results. Transects were already partially invaded when established, thus time since invasion is an unknown factor that may have influenced the invasion dynamics observed in the two lakes. In addition, the datasets from Koronis and Moose are not perfect complements due to inconsistencies in sampling. These should be considered when comparing results from the two lakes. Koronis had three years of complete data and one year of partial data (year 2). Moose had two years of complete data and one year of partial data (year 3). However, transects in both lakes were fully sampled in year 1 and 4 which justifies general comparisons of initial and final conditions. It is also worth noting that a subset of transects in Moose were

not resampled in year 3 due to extremely dense and tall growth of *Nitellopsis*. In year 4, *Nitellopsis* growth seemed to subside somewhat and these transects were relocated. Thus, a period of peak *Nitellopsis* growth in Moose was missed in the dataset.

Effects of *Nitellopsis* invasion on native species are likely to be dynamic over time due to annual variation in *Nitellopsis* abundance and the strong relationship between abundance and impacts. Large temporal variation in biomass levels is typical of charophytes, and of annual species in general (De et al. 2013). While there was still a net loss in diversity over the study period in Koronis, the resurgence of native species in year 4 was surprising and may suggest some resilience of native communities to the effects of *Nitellopsis* invasion. *Nitellopsis* has only been known in Minnesota since 2015; native species could show a strong negative response to *Nitellopsis* invasion initially but recover over time. Acute impacts early on in an invasion that wane over longer time scales have been documented in other biological invasions (Strayer et al. 2006). While the immediate impacts of *Nitellopsis* invasion documented here may not remain consistent over time, short-term impacts on native plants should not be minimized and still raise concerns for biodiversity and habitat management. Factors responsible for temporal variation in abundance of *Nitellopsis* are not currently well understood.

Between the two study lakes, there were notable differences in *Nitellopsis*–native plant dynamics, providing an interesting case study on invasion impacts. Koronis transects were rapidly invaded over a three-year period and *Nitellopsis*

peaked in abundance while native species nearly vanished; however, there was a rebound in the native community in year 4. Moose transects saw steady expansion of *Nitellopsis* over the four-year period but maintained moderate growth levels, while the native community was preserved over the four-year period.

The severity of *Nitellopsis* invasion and magnitude of impacts will vary between systems. *Nitellopsis* invasion seems to be more severe in a lower-diversity, somewhat impaired (slightly eutrophic) system like Koronis. *Nitellopsis* expanded faster and reached higher abundances in Koronis compared to Moose. Lower biodiversity and/or ecological impairment resulting in greater invader dominance is a consistent theme in invasion biology (Zedler and Kercher 2004, Schaffelke et al. 2006, Capers et al. 2007). Moose had higher water clarity and species diversity than Koronis, and *Nitellopsis* was not as abundant, which could explain the lower impacts in this system. The differences in *Nitellopsis* invasion patterns between lakes could also be due to differences in environmental conditions or time since invasion, since biotic resistance may not strongly influence macrophyte invasions in aquatic systems (Capers *et al.*, 2007; Muthukrishnan, Hansel-Welch & Larkin, 2018).

The high rate at which *Nitellopsis* was able to expand and displace native macrophytes was a surprising result of this study. Displacement of native species has been well-documented for some aquatic invasive plants, but this study provided the strongest evidence of this impact to date for *Nitellopsis*. The rapid expansion of *Nitellopsis* motivates the need to identify new infestations early and target

management to prevent lake-wide infestations. Invasion patterns presented here were over a short-term period (a maximum of four years). *Nitellopsis* was only recently discovered in Minnesota and may be in an early, rapidly accelerating phase of its invasion. It is unknown whether *Nitellopsis* will continue to increase at the same rate or slow into a standoff with native macrophytes over time. Boylen et al. (1999) showed native suppression persisted with *Myriophyllum spicatum* invasion over a 10-year sampling period. Impacts of *Typha* × *glauca* increased as invasions aged across decadal time scales (Mitchell et al. 2011). Revisiting TSS transects in the future could provide valuable insights on long-term outcomes of invasion, and whether natives are able to recover over time.

Nitellopsis can displace high-value native species, which is of concern for biodiversity, habitat quality, and ecosystem services. Of particular concern is the ability of *Nitellopsis* to invade and replace native charophyte-dominated habitat. The carpet-forming group (*Chara* spp. and *Najas* spp.) is the most functionally similar to *Nitellopsis* and was among the most sensitive, which suggests niche overlap that may increase potential for competitive exclusion (Godoy 2019, Verhoeven et al. 2020). Native charophytes are a diverse group that provide high quality habitat and water quality benefits (Kufel and Kufel 2002). It is unknown whether *Nitellopsis* contributes ecosystem services at the same level as native charophytes; declines in *Chara* spp. due to *Nitellopsis* are, at minimum, a concern for conservation of native biodiversity. *Nitellopsis* is likely to have significant community-level effects in charophyte-dominated lakes, and these populations

should be closely monitored where *Nitellopsis* occurs. Additionally, control of *Nitellopsis*, which is predominantly done using copper-based algaecides, has the potential for non-target impacts to native charophytes, and this risk should be weighed against invasion impacts.

Despite the spatial and temporal variability of *Nitellopsis* documented in this study, there were consistent trends in impacts that are important to highlight. The carpet-forming group, most functionally similar to *Nitellopsis*, declined sharply in both lakes and did not recover like other groups, such as pondweeds. Declines in carpet-forming *Najas* spp. were among the only negative changes in the native community in Moose over the study period. Losses of charophytes and *Najas* spp. as *Nitellopsis* increases should be expected and may persist longer than reductions of other native taxa. Lastly, where *Nitellopsis* is successful and abundance is consistently high, impacts will persist, and native species may lose the ability to recover over time due to degradation of seed and propagule banks.

In summary, my hypotheses that *Nitellopsis* would be associated with reductions of native macrophytes and changes to diversity, species composition, and functional composition of invaded assemblages were supported. *Nitellopsis* reduced species richness and native cover and was associated with changes to the composition of macrophyte assemblages across the three study lakes. Competitive displacement is likely to be a significant component of declines in native species where *Nitellopsis* occurs; however, experimental work is needed to draw conclusions about its mechanisms of dominance. *Nitellopsis* invasion is likely to

lead to increasingly homogenous and less-diverse vegetation over time, which could have implications for other aquatic organisms and ecosystem services. Outcomes of *Nitellopsis* invasion will vary between lakes, but where and when *Nitellopsis* is successful, rapid expansion and declines in native macrophytes can occur, which is concerning from a conservation standpoint. Further investigating conditions associated with *Nitellopsis* growth could be valuable for better explaining apparent differences in invasiveness across space and time that were observed in this study.

Nitellopsis is the first invasive macroalga in Minnesota and poses a novel threat to aquatic plant communities and potentially other aquatic organisms. *Nitellopsis* is an aggressive, high-impact invader and spread prevention and management of this AIS should be prioritized. Identifying infestations early on is critical because *Nitellopsis* can grow and expand quickly. Hand-pulling of small infestations offers targeted control with minimal disturbance, while management at larger scales results in greater disturbances, which *Nitellopsis* can potentially benefit from—synergy between passenger and driver phenomena that has been dubbed "back-seat driver" effects (Bauer 2012). This is especially true since control strategies for *Nitellopsis* have not shown widespread or lasting suppression to date. My research demonstrated that native charophytes are particularly sensitive to *Nitellopsis* invasion; however, copper algaecides used to control *Nitellopsis* can also harm native charophytes. Potential non-target impacts of management should be weighed against the ecological impacts of invasion to make sound management decisions. The significant impacts of *Nitellopsis* observed in this study suggest that

failure to contain and manage its spread could have significant negative consequences for aquatic plant communities in Minnesota lakes.

Table 2.1 Summary of field sampling efforts to assess plant community impacts of *Nitellopsis*

Year/Lake	Collection Method
2017 – Koronis	Initial sampling of TSS transects (*N* = 15)
2017 – Moose	Initial sampling of TSS transects (*N* = 15)
2018 – Koronis	Sampled SFTS transects (*N* = 25); Partially resampled TSS transects (*n* = 4)
2018 – Moose	Sampled SFTS transects (*N* = 25)
2018 – Winnibigoshish	Sampled SFTS transects (*N* = 17)
2019 – Koronis	Resampled TSS transects (*N* = 15)
2019 – Moose	Partially resampled TSS transects (*n* = 9)
2020 – Koronis	Resampled TSS transects (*N* = 15)
2020 – Moose	Resampled TSS transects (*N* = 15)

Study lakes for space-for-time substitution (SFTS) sampling were Koronis, Moose, and Winnibigoshish; study lakes for time sequence sampling (TSS) were Koronis and Moose. TSS transects were first established and sampled in 2017, '*n*' indicates a partial resampling of the full set of TSS transects (*N*). SFTS transects were non-permanent and sampled at a single point in time.

Table 2.2 Summaries of GLMMs for native macrophyte richness and cover using SFTS data.

	Log-Mean	SE	*z* value	*P*
Species Richness				
Nitellopsis cover	-0.04	0.007	-6.04	< **0.001**
Lake Koronis	0.85	0.08	9.64	< **0.001**
Moose Lake	1.30	0.08	14.77	< **0.001**
Lake Winnibigoshish	1.44	0.10	13.66	< **0.001**
Native Cover				
Nitellopsis cover	-0.18	0.005	-31.06	< **0.001**
Lake Koronis	2.12	0.04	48.23	0
Moose Lake	2.07	0.04	48.42	0
Lake Winnibigoshish	1.93	0.05	34.33	< **0.001**

Both GLMMs assumed a Poisson error distribution with a log link function. Coefficients are untransformed and on the log scale. Significant predictors in bold text.

Table 2.3 Univariate generalized linear models testing species-specific impacts of *Nitellopsis* cover using SFTS data.

Species	*Intercept*	*Slope*	*P*
Bidens beckii	-2.95	-0.14	0.98
Ceratophyllum demersum	-0.33	-0.11	0.17
***Chara* sp.**	**1.37**	**-0.17**	**< 0.001**
Elodea canadensis	-1.65	-0.08	0.92
Heteranthera dubia	-2.67	-0.25	0.98
Hippuris vulgaris	-6.04	0.09	0.98
Lemna trisulca	-2.24	-0.38	0.77
Myriophyllum sibiricum	-1.20	-0.09	0.38
Najas flexilis	-0.84	-0.04	0.98
Najas guadalupensis	**0.99**	**-0.24**	**< 0.001**
Nuphar variegata	-8.19	0.42	0.98
Nymphaea odorata	-5.12	-0.08	0.98
Potamogeton amplifolius	-2.20	-0.05	0.98
Potamogeton foliosus	-6.35	0.30	0.96
Potamogeton friesii	-1.70	-0.03	0.98
Potamogeton gramineus	-3.02	-0.06	0.98
Potamogeton illinoensis	**-1.73**	**-0.35**	**< 0.001**
Potamogeton natans	-3.23	-0.11	0.98
Potamogeton nodosus	-3.36	-0.24	0.95
Potamogeton praelongus	-1.13	-0.24	0.95
Potamogeton pusillus	-1.90	-0.10	0.17
Potamogeton richardsonii	-1.32	-0.23	0.17
Potamogeton strictifolius	-4.00	-0.19	0.98
Potamogeton zosteriformis	-1.24	-0.05	0.98

Ranunculus aquatilus	-4.51	-0.43	0.98
Saggitaria sp.	-4.63	-0.25	0.98
Stuckenia pectinata	**-0.45**	**-0.13**	**0.01**
Utricularia macrorhiza	-1.69	-0.11	0.35
Utricularia minor	-10.18	0.50	0.98
Vallisneria americana	**-0.32**	**-0.19**	**< 0.001**

Coefficients shown are untransformed and on the log-scale. Species exhibiting significant responses to *Nitellopsis* cover in bold text.

Table 2.4 Univariate generalized linear models testing responses of functional groups to *Nitellopsis* cover using SFTS data.

Group	*Intercept*	*Slope*	*P*
Basal	**-0.22**	**-0.19**	**< 0.001**
Carpet	**1.95**	**-0.16**	**< 0.001**
Dissected, rooted	**-1.00**	**-0.09**	**0.04**
Dissected, unrooted	**-0.11**	**-0.11**	**< 0.001**
Floating pondweeds	-2.62	-0.10	0.48
Small, unrooted	**-2.34**	**-0.33**	0.43
Submersed pondweeds, broad	**-0.40**	**-0.16**	**< 0.001**
Submersed pondweeds, narrow	**0.37**	**-0.10**	**< 0.001**

Coefficients are untransformed and on the log-scale. Functional groups exhibiting significant responses to *Nitellopsis* cover in bold text.

Table 2.5 GLMMs fit to evaluate changes in presence of *Nitellopsis* and native vegetation over time on TSS transects.

Response:	***Nitellopsis* presence**				**Native macrophyte presence**			
Lake Koronis								
	Log-odds (logit)	SE	z value	*P*	Log-odds	SE	z value	*P*
(Intercept)	**2.79**	**1.13**	**2.45**	**0.014**	**5.66**	**1.03**	**5.52**	**< 0.001**
year 3	**2.96**	**0.44**	**6.78**	**< 0.001**	**-5.87**	**1.02**	**-5.78**	**< 0.001**
year 4	**3.66**	**0.52**	**7.02**	**< 0.001**	**-3.19**	**1.02**	**-3.10**	**0.002**
Moose Lake								
(Intercept)	-0.62	0.37	-1.67	0.10	**3.29**	**0.38**	**8.55**	**< 0.001**
year 3	**1.27**	**0.31**	**4.10**	**< 0.001**	-0.85	0.52	-1.65	0.10
year 4	**2.04**	**0.27**	**7.42**	**< 0.001**	2.00	1.07	1.87	0.06

Table showing results from four models; all models assumed a binomial error distribution with a logit link function. Top and bottom panels show model results from Lake Koronis and Moose Lake, respectively. Left and right panels of the table show the models for the two responses investigated, *Nitellopsis* presence and native macrophyte presence. Model coefficients and associated *p*-values are shown, with significant predictors in bold text; coefficients are untransformed and on the log-odds scale (logit). Models are effects coded, with intercepts representing year-one conditions.

Table 2.6 GLMMs fit to analyze vegetation changes over time on TSS transects.

Response:	***Nitellopsis* cover**				**Species richness**			
Lake Koronis								
	Log-Mean	SE	z value	*p*	Log-Mean	SE	z value	*p*
(Intercept)	1.13	0.09	12.75	< **0.001**	0.92	0.08	10.99	< **0.001**
year 2	0.48	0.07	6.80	< **0.001**	-0.06	0.13	-0.46	0.64
year 3	0.75	0.05	16.46	< **0.001**	-0.62	0.11	-5.60	< **0.001**
year 4	0.61	0.05	13.03	< **0.001**	0.21	0.08	2.62	< **0.001**
Nitellopsis	NA	NA	NA	NA	-0.11	0.01	-7.66	< **0.001**
Moose Lake								
(Intercept)	0.65	0.13	5.03	**<0.001**	1.15	0.08	14.69	< **0.001**
year 2	NA	NA	NA	NA	NA	NA	NA	NA
year 3	0.61	0.08	7.80	< **0.001**	0.24	0.07	3.39	< **0.001**
year 4	0.71	0.06	11.97	< **0.001**	0.72	0.06	12.83	< **0.001**
Nitellopsis	NA	NA	NA	NA	-0.08	0.01	-8.38	< **0.001**

Table showing results from four models fit to TSS data; all models assumed a Poisson error distribution with a log link function. Top and bottom panels show model results from Lake Koronis and Moose Lake, respectively. Left and right panels of the table show the models for the two responses investigated, *Nitellopsis* cover and species richness, respectively. Model coefficients and associated *p*-values are shown, with significant predictors in bold text; coefficients are untransformed and on the log scale. Models are effects coded, with intercepts representing year-one conditions. NA indicates that variable not included in the model.

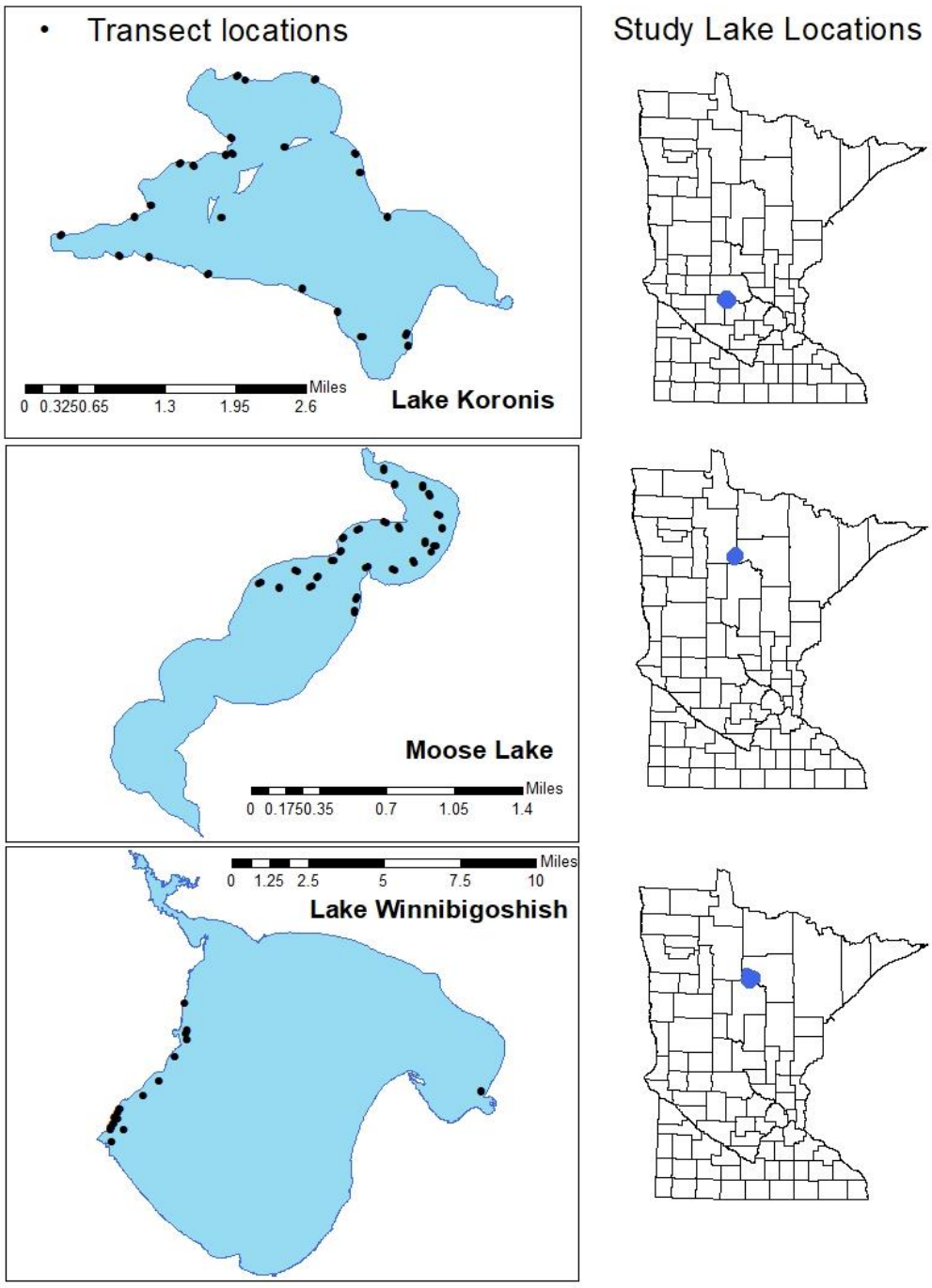

Figure 2.1 Study lakes and sampling locations of SFTS transects. Transects were distributed throughout the known extent of *Nitellopsis* in each lake and sampled during the 2018 field season. Note the differing scales for each lake; transects may

appear close together due to differing map scales but were always >75-m apart from one another. Moose Lake and Lake Winnibigoshish are ~20 km apart.

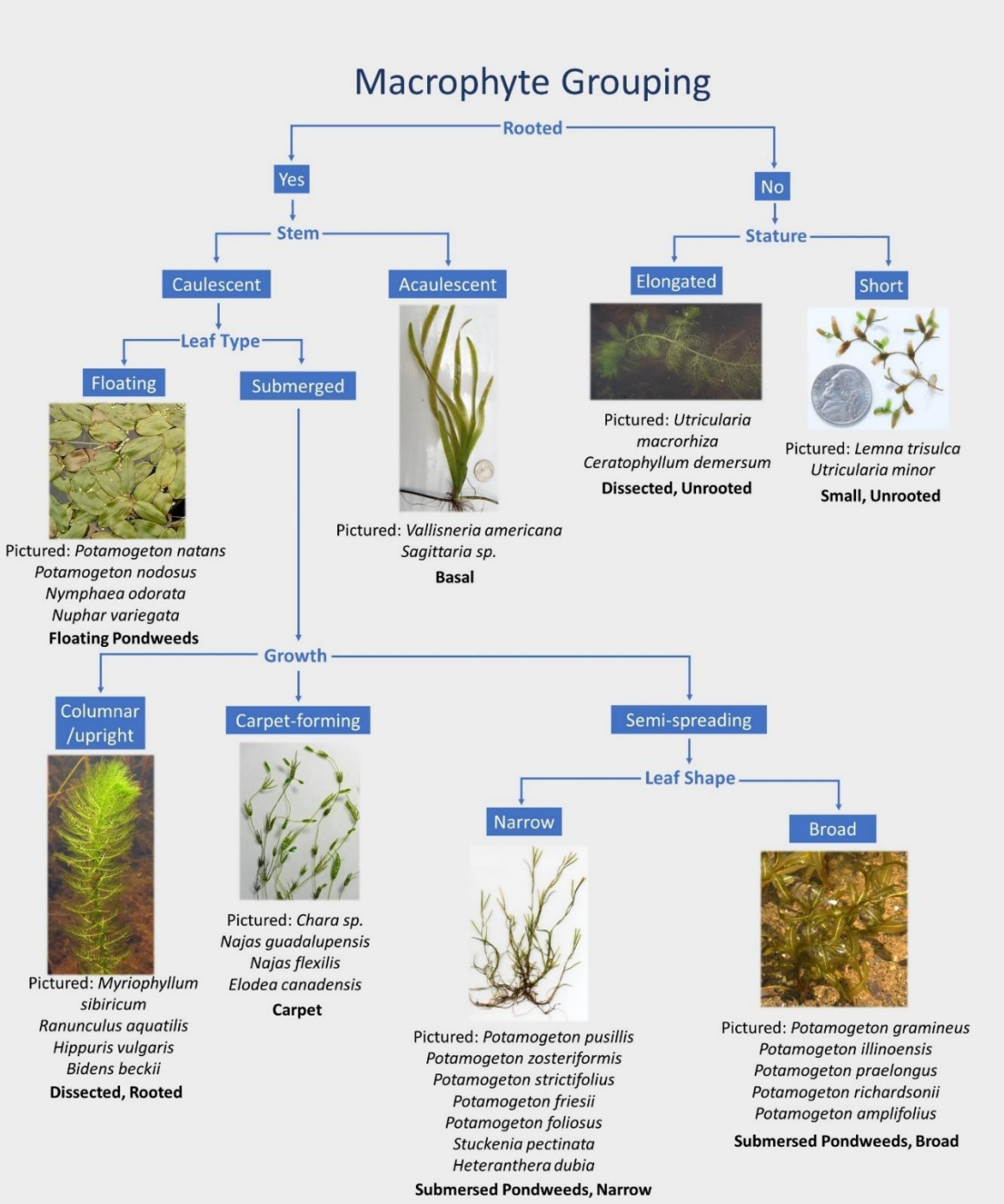

Figure 2.2 Decision tree and resulting categories for native macrophyte functional group classifications. Group names are bolded.

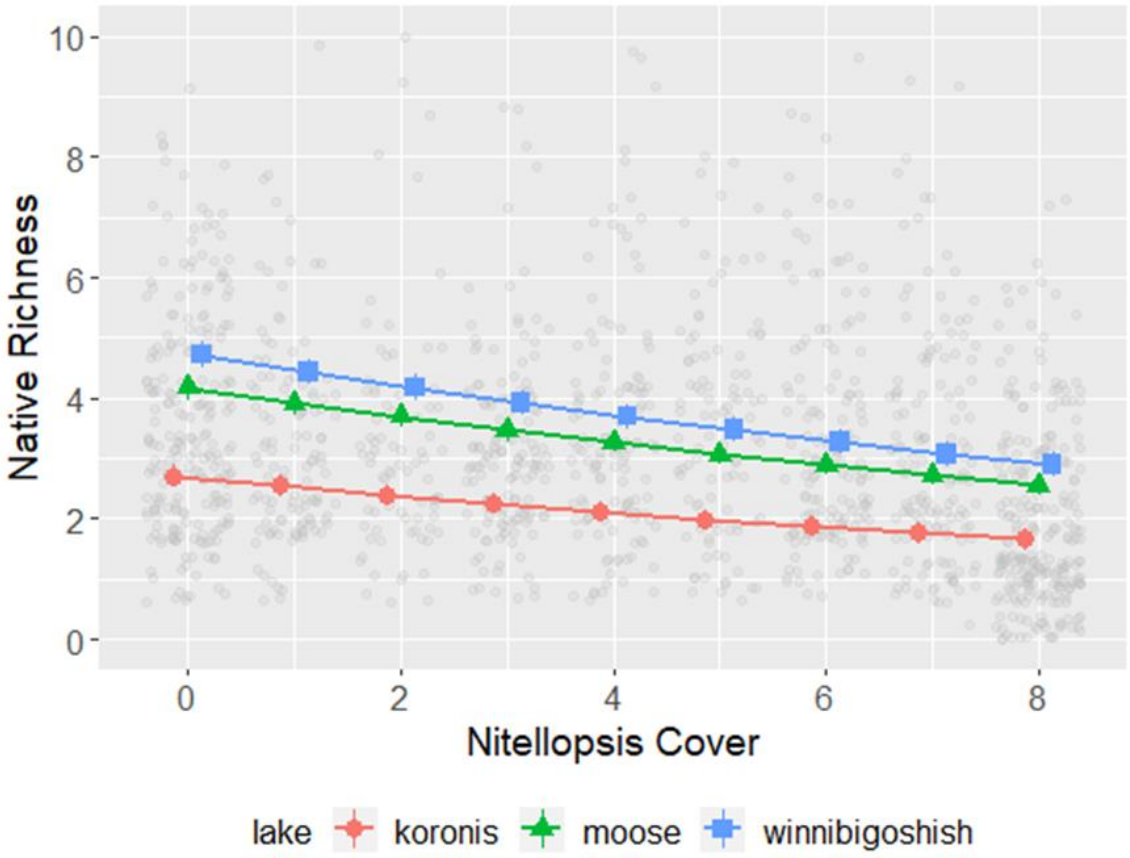

Figure 2.3 Relationship between native macrophyte richness and *Nitellopsis* cover. Figure shows results from native richness GLMM with a Poisson error distribution fit to SFTS data. Lines show model fit. Opaque gray circles represent observed values (quadrats).

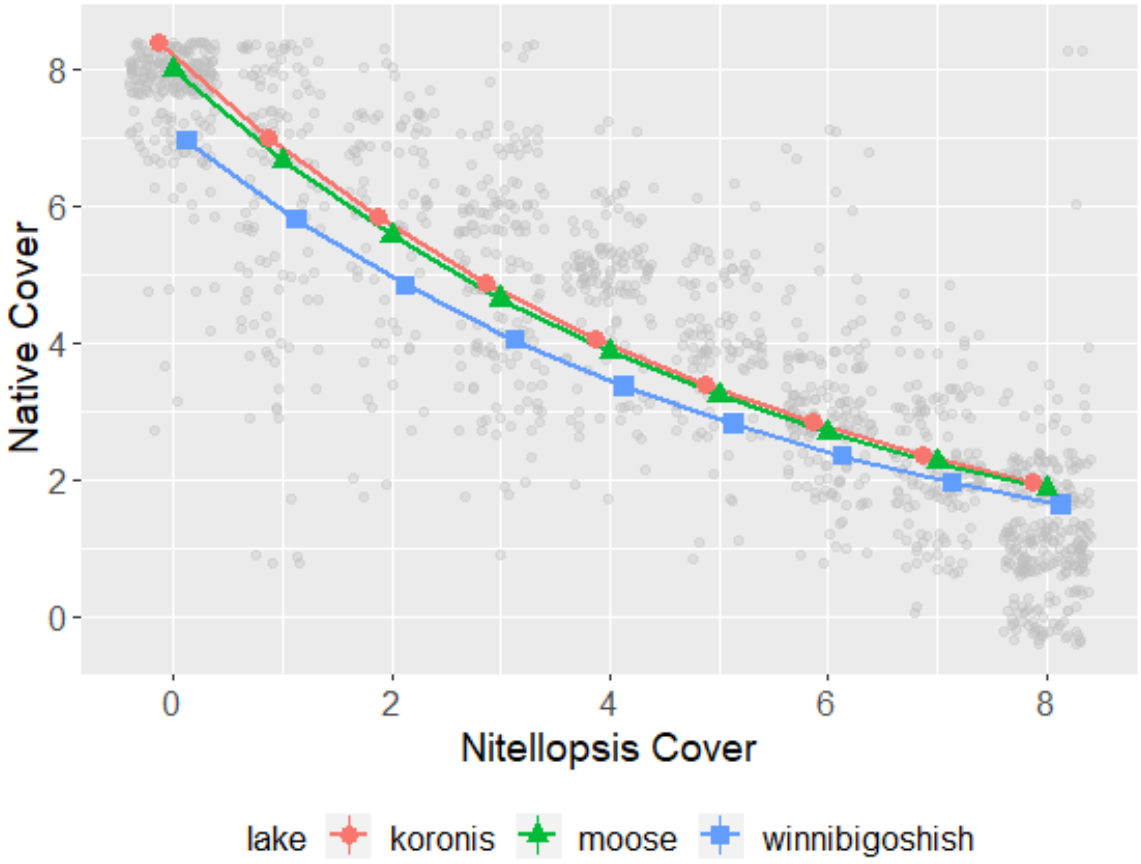

Figure 2.4 Relationship between native macrophyte cover and *Nitellopsis* cover. Figure shows results from native richness GLMM with a Poisson error distribution fit to SFTS data. Lines show model fit. Opaque gray circles represent observed values (quadrats).

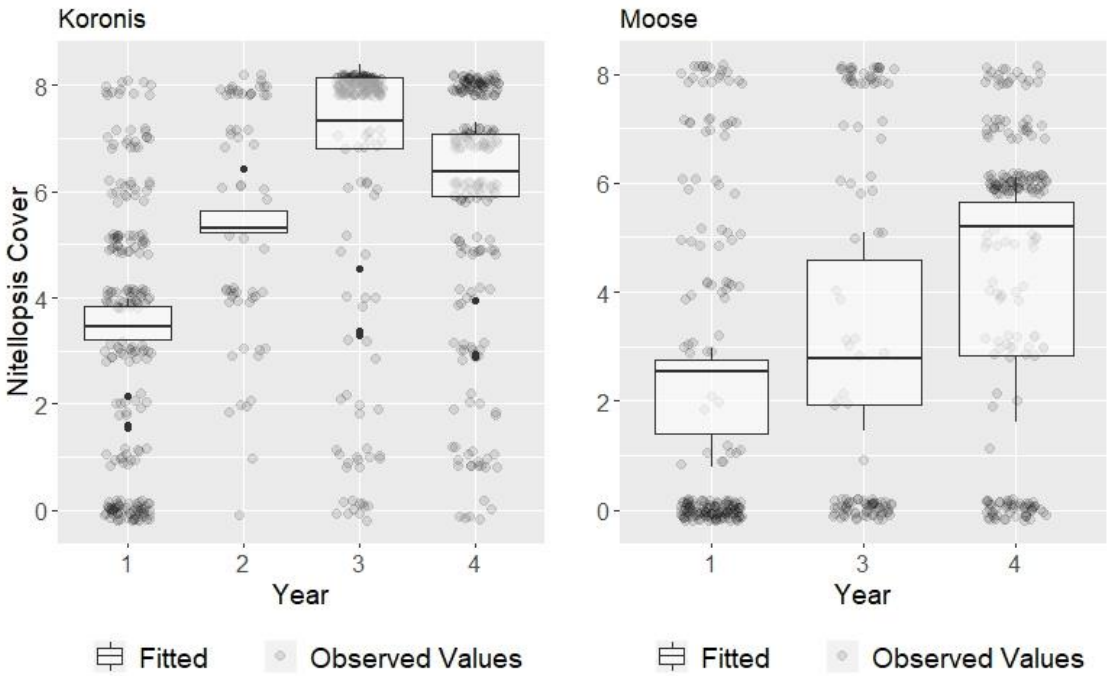

Figure 2.5 *Nitellopsis* cover over time on TSS transects. Figure shows results of individual GLMMs fit for each lake. Boxplots show model predictions and opaque gray circles represent observed values (quadrats). In Koronis, only 4 out of 15 transects were resampled in year 2. In Moose, no transects were resampled in year 2; 9 out of 15 were resampled in year 3.

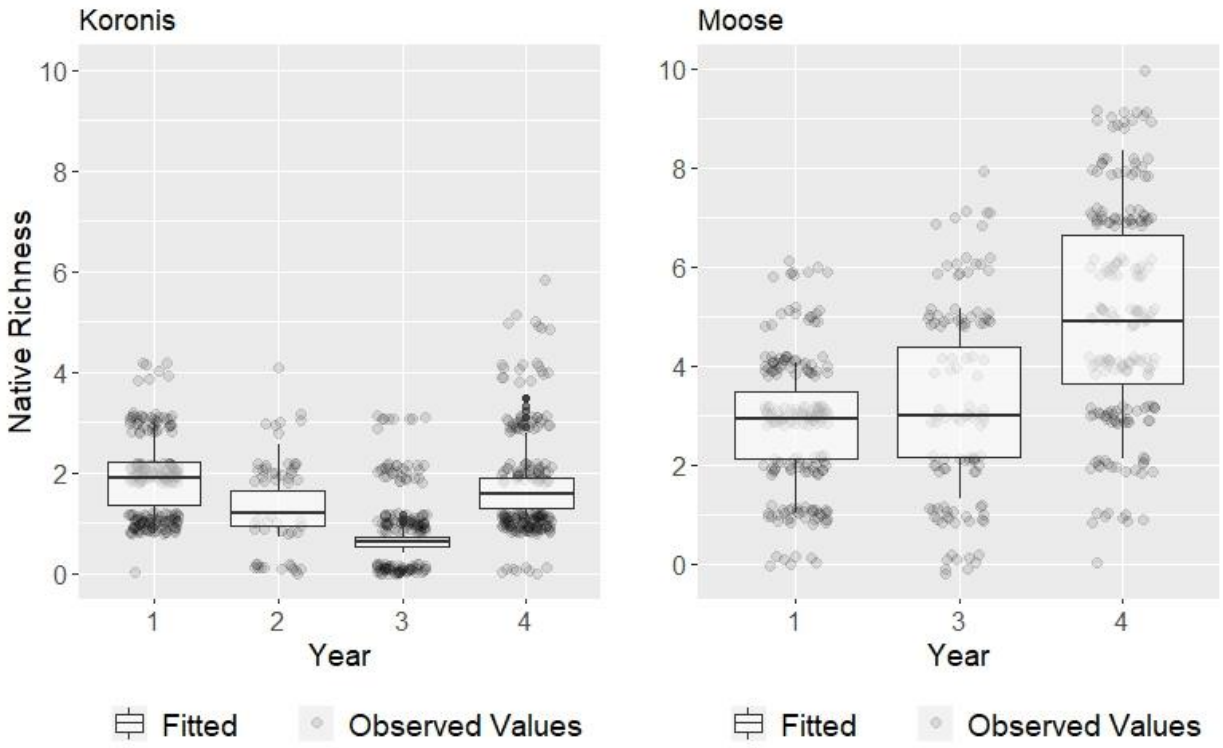

Figure 2.6 Native macrophyte richness over time on TSS transects. Figure shows results of individual GLMMs fit for each lake. Boxplots show predictions and opaque gray circles represent observed values (quadrats). In Koronis, only 4 out of 15 transects were resampled in year 2. In Moose, no transects were resampled in year 2; 9 out of 15 were resampled in year 3.

Chapter 3

Environmental preferences of *Nitellopsis obtusa* at fine spatial scales – investigating conditions associated with nuisance growth

Summary

Nuisance (i.e., excessive or prolific) growth is the root of many problems associated with invasive aquatic plants (e.g., ecological and recreational impacts, limited effectiveness of control measures). Environmental conditions regulate plant growth, thus, understanding of environmental preferences and tolerances of invasive aquatic plants can be used to predict areas of greater vulnerability to invasion. Broad-scale environmental factors associated with presence of the invasive macroalga *Nitellopsis obtusa* have been investigated, aiding surveillance and early detection efforts. However, there is limited understanding of the fine-scale environmental preferences of *Nitellopsis obtusa*. This leaves uncertainty regarding how broadly *Nitellopsis obtusa* may be able to reach nuisance growth levels and limits our ability to anticipate where impacts are likely to be most severe. I investigated the environmental preferences of *Nitellopsis obtusa* at fine spatial scales and explored whether any conditions were associated with nuisance growth. In three Minnesota lakes, I collected data on *Nitellopsis* cover and associated water depth, total organic carbon, total nitrogen, phosphorus, and sediment texture. In general, *Nitellopsis* cover did not show significant responses to the environmental variables measured, aside from an unexpected, modest negative response to increasing nitrogen. It is uncertain whether this is indicative of broad environmental tolerances of *Nitellopsis,* or

if there are conditions influential to its growth that were just not captured by my study design. The fine-scale environmental preferences of *Nitellopsis* remain largely undefined. Environmental tolerances are a key component of threat assessments and more research is needed to address this gap for *Nitellopsis*.

Introduction

Non-native aquatic plants can be a nuisance in public waters and pose ecological threats to aquatic ecosystems. Human-assisted movement has removed barriers between waterbodies and led to rapid spread and establishment of non-native aquatic plants. In some cases, new invaders have emerged so quickly that management responses have been implemented despite little knowledge of the biology or ecology of the target species (e.g., for *Nitellopsis obtusa*, starry stonewort, in the Great Lakes region). Invasive aquatic plant management requires significant time and money, and strategies grounded in knowledge of the target species can maximize return on investment of limited resources. One key area of research is defining the ecological niche and preferences of an invasive species (Simberloff et al. 2005). Determining what habitats an invader survives and thrives in answers two questions for resource managers: *where might an invasive species occur and where is it likely to cause the greatest damage?* This information can guide early detection efforts and prioritization of management efforts.

There is often a perception that invasive species will eventually come to dominate any environment they are introduced to (Larson 2005); however, this is not necessarily the case. For example, in a comparison of invasive and native aquatic plants in Wisconsin, both were generally found to occur at low densities, with high-density populations the exception – described as ‘commonly rare, rarely common’ (Hansen et al. 2013). Nonetheless, when invasive species do become abundant, they tend to reach higher densities than native species (Powell et al. 2011, Hansen et al. 2013). Impacts scale with invader density (Kumschick et al. 2015), thus, the more dominant an invader

becomes in an area, the greater the possible ecological harm. Ecological impacts are not the only concern when invaders peak in abundance. Management effort and costs increase with abundance (Yokomizo et al. 2009), for example, higher pesticide application rates may be needed or physical removal methods may take longer. Densely invaded areas also have higher propagule density and potential to serve as sources of new infestations (Von Holle and Simberloff 2005). Invasions are often treated as binary – is a given habitat invaded or uninvaded? – but abundance is a key aspect that affects both the consequences of an invasion and subsequent management responses (Catford et al. 2012). Hansen et al (2013) emphasized that heterogeneity in invasive plant abundance, and factors responsible for the variation, should receive more attention in invasive species prevention and management. Environmental conditions have a significant influence on macrophyte abundance (Mikulyuk et al. 2011); thus, identifying the environmental associations of invasive aquatic plants, particularly conditions that promote nuisance growth, is an important component of applied research.

In general, invasive aquatic plants tend to have broad environmental tolerances (Zedler and Kercher 2004, Higgins and Richardson 2014), enabling them to become dominant across variable environmental conditions. Invaders that are more tolerant of a wide range of conditions are likely to become greater problems across the landscape (Evangelista et al. 2008). However, while invasive plants may be able to establish widely due to broad environmental tolerances, there is often a suite of optimal conditions that stimulate the most severe invasions. For example, *Myriophyllum spicatum* can be found in low to high nutrient systems, but nuisance growth is concentrated in fertile areas

(Smith and Barko 1990). Understanding the factors that promote nuisance growth and ecological impacts is important for invasion response and management.

The macroalga *Nitellopsis obtusa* (hereafter *Nitellopsis*), is rare or endangered across most of its native range in Europe and Asia, but in its introduced range in North America, observations of dense infestations have become increasingly common in recent years. Habitat degradation and poor water quality have been cited as drivers of *Nitellopsis* declines in its native range (Larkin et al. 2018). Environmental conditions found in northern tier U.S. lakes, where *Nitellopsis* is expanding, appear to be more favorable. Indeed, colonization of new environmental (climatic) niche space following introduction to North America represents a potentially beneficial expansion of the realized niche of *Nitellopsis* in its invaded range, which may help explain its transition from a rare native to a dominant invasive (Escobar et al. 2016).

In Chapter 2, I demonstrated that *Nitellopsis* is a significant threat to native aquatic plant communities. Invasion impacts were most significant at the highest levels of *Nitellopsis* abundance (i.e., approaching 100% cover). Such nuisance growth levels also impede access and use of public waters (Harman and Albright 2012, Sleith et al. 2015). In the most heavily invaded areas, *Nitellopsis* can completely fill the water column (Pullman and Crawford 2010). Since impacts of *Nitellopsis* are correlated with its abundance, it is important to know what conditions promote *Nitellopsis* abundance. Identifying relationships between *Nitellopsis* and its environment could provide a basis for better predicting ecological risk and determining where to focus control efforts. However, factors responsible for *Nitellopsis* abundance at local scales are not well

understood. There is little understanding of how *Nitellopsis* responds to heterogeneous environmental conditions *within* lakes, for example, which is especially pertinent as *Nitellopsis* abundance can be highly variable across space and time. Will *Nitellopsis* eventually come to dominate littoral zones in invaded lakes? Or are there environmental limits on nuisance growth of *Nitellopsis* likely to leave some lakes or some areas within lakes relatively unimpacted? These remain key unanswered questions regarding the ecology and future spread and impacts of *Nitellopsis*.

Nitellopsis is in an early invasion stage in the Upper Midwest and appears to be expanding rapidly (Larkin et al. 2018). In response, there has been recent research to inform spread risk. Previous work has focused on using environmental conditions associated with *Nitellopsis* presence to predict invasion risk at broad spatial scales (continental and regional). In general, this research has identified high risk of *Nitellopsis* expansion and underscores the need to better understand the basic ecology and growth of this invader. A climate-based niche model showed that large regions of North America have conditions suitable for *Nitellopsis* establishment (Escobar et al. 2016); this is important for threat assessment of future *Nitellopsis* spread at a coarse scale. However, climate has a limited influence on aquatic plants except at large geographic or bioclimatic scales because water is buffered from the extreme temperature changes that terrestrial systems are exposed to (Bornette and Puijalon 2011). The growth and distribution of aquatic plants is instead more influenced by within-lake conditions (e.g., water and sediment chemistry) (Santamaría 2002).

Additional work has focused on how lake-level environmental conditions influence the presence/absence of *Nitellopsis*. Reported patterns include invaded lakes tending to have elevated pH, conductivity, and nutrients (Sleith et al. 2015, Midwood et al. 2016, Muthukrishnan et al. 2018b). In Minnesota and Wisconsin lakes, there appears to be widespread suitability for *Nitellopsis* establishment, particularly in mesotrophic to eutrophic, calcareous waters (Muthukrishnan et al. 2018b). Thus, there is emerging understanding of the broad types of lakes that are suitable for *Nitellopsis*, which is important for early detection and monitoring strategies. However, within-lake assessments of *Nitellopsis* growth are needed, as natural resource management and protection tend to be implemented at the scale of individual lakes; for example, at the behest of a lake association working in conjunction with agency resource professionals.

There is little information available on how local, fine-scale environmental conditions influence *Nitellopsis* abundance. However, studies of other characean algae provide some basis for inferring what *Nitellopsis*' environmental preferences might be. Water depth often has a strong influence on charophyte growth because it is correlated with light availability, exposure to wave action, and thermal conditions (Berg 1999). Tall charophytes, such as *Nitellopsis*, are sensitive to wave disturbance and are more likely to occur in deeper water. In general, charophytes prefer soft substrates with finer particle sizes characterized by higher silt and clay or silt and organic matter (Kovtun-Kante 2015). Thus, sediment texture may be an important factor in *Nitellopsis* growth. Sediment chemical composition also mediates charophyte growth; in particular, organic carbon,

nitrogen, and phosphorus concentrations have been identified as regulating factors for charophyte growth (Rojo et al. 2019).

To date, only one paper has reported on environmental conditions associated with *Nitellopsis* abundance at fine spatial scales, in this case to explain variation within an infested reservoir in Ontario (Harrow-Lyle & Kirkwood, 2020). Beginning with 12 candidate variables related to habitat and water chemistry, Harrow-Lyle and Kirkwood (2020) found water depth and total nitrogen to be the only significant variables explaining *Nitellopsis* abundance. Water depth was positively correlated with *Nitellopsis* abundance, although their range of observations only spanned 1-2 m, while *Nitellopsis* has been reported in water depths up to 7 m (Larkin et al. 2018). Harrow-Lyle and Kirkwood (2020) did not detect significant relationships between *Nitellopsis* abundance and variables found to be important to its broad-scale (lake-level) distribution in other studies (e.g., pH and conductivity), which could be because within-lake variability in water chemistry may be insufficient to produce detectable differences in growth, i.e., due to mixing, water chemistry may not be sufficiently localized to explain variation in local abundance. In contrast, sediment conditions within lakes can be highly heterogeneous and relatively stable within a location, and have been shown to influence the abundance of other invasive macrophytes (Hoffmann et al. 2013).

Sediment characteristics have been suggested as potentially important predictors of *Nitellopsis* abundance; however, this relationship has not been rigorously described. There are observations suggesting *Nitellopsis* prefers rich, fine-textured sediment (Schloesser et al. 1986, Midwood et al. 2016, Larkin et al. 2018). As a charophyte,

Nitellopsis lacks true roots, and finer substrates may provide better anchorage for rhizoids. Counter to this expectation, *Nitellopsis* was reported to be most robust in areas of marl or sand in several inland Michigan lakes (Alix et al. 2017). However, Midwood et al (2016) observed that *Nitellopsis* seemed to prefer finer sediments and was uncommon in sandy areas. Additionally, nutrient availability in sediments may be important for *Nitellopsis* growth; charophytes acquire nutrients from both the sediment via rhizoids and directly from the water column (Vermeer et al. 2003, Asaeda et al. 2008). Associations between *Nitellopsis* and sediment conditions need to be quantified to better understand drivers of its growth.

The goal of this work was to improve understanding of the drivers of *Nitellopsis* abundance at fine spatial scales, and to identify environmental characteristics that might be associated with high invasion severity. This study complements larger-scale analyses of environmental parameters associated with *Nitellopsis'* regional distribution (lake-level presence/absence), which can be used to support risk assessment and detection efforts. Additionally, this study is the first to investigate sediment conditions associated with variation in *Nitellopsis* abundance in multiple lakes, despite sediment characteristics having been invoked as important for 10+ years based on anecdotal observations. I collected data from the space-for-time-substitution (SFTS) transects introduced in Chapter 2 to assess relationships between *Nitellopsis* abundance and a suite of environmental conditions known to be important for charophyte growth: water depth, total organic carbon, total nitrogen, phosphorus, and sediment texture. This objective was

implemented to improve the capacity to predict where *Nitellopsis* will be most problematic in order to inform expectations of impacts and guide management responses.

Methods

Field work for this study occurred in July and August of 2018, in three Minnesota lakes containing *Nitellopsis*: Koronis (Stearns Co.), Moose (Beltrami Co.), and Winnibigoshish (Cass/Itasca Co) (Figure 2.1). Koronis is a slightly eutrophic lake located in central Minnesota; Moose and Winnibigoshish are mesotrophic lakes located in northern Minnesota. All three study lakes have broadly dispersed, well-established populations of *Nitellopsis*. See Chapter 2 for full descriptions of study lakes.

The environmental sampling described here was performed using the SFTS transects detailed in Chapter 2. In brief, transects were set up along gradients of *Nitellopsis* abundance (i.e., vegetation transition from invaded to uninvaded and high to low *Nitellopsis* density). Transects were placed on the lake bottom and sampled using SCUBA-based surveying to enable assessment of fine-scale relationships between environmental conditions and *Nitellopsis* abundance. There were 26 transects in Koronis, 25 in Moose, and 17 in Winnibigoshish.

Water chemistry parameters were measured once in each transect. Only a single measurement was taken because local water chemistry was not expected to systematically vary within the 20-m transects. These measurements were not paired with a single corresponding measure of *Nitellopsis* abundance as they were measured at the site (transect) level, rather than the plot level at which *Nitellopsis* abundance was observed.

Measurements were taken using a YSI Incorporated Professional Plus Multiparameter Instrument (YSI Inc.; Yellow Springs, OH). The probe was lowered 1-m into the water and allowed to reach equilibrium before recording measurements of pH, conductivity, and dissolved oxygen (mg/L). In total, there were 68 water chemistry observations (one per transect).

Sediment and water depth were subsampled along transects and paired with cover-based measures of *Nitellopsis* abundance within 1-m^2 quadrats. Specifically, in three quadrats per transect, environmental data were collected along with vegetation data (hereafter 'environmental quadrats'). Environmental quadrats were selected to represent high (>75% cover), medium (25-75%) and low (<25%) *Nitellopsis* cover from each transect. Transect end points (quadrats at 0-m and 19-m) were most often used for environmental quadrats representing high and low *Nitellopsis* cover, respectively. The third environmental quadrat was placed at a position along the transect best representing intermediate *Nitellopsis* abundance. In each environmental quadrat, water depth was recorded and sediment cores were collected. These data were paired with estimates of *Nitellopsis* cover (see Chapter 2) from the same environmental quadrats to explore associations between environmental conditions and *Nitellopsis* abundance. There were a total of 204 environmental quadrats from Koronis (n = 78), Moose (n = 75), and Winnibigoshish (n = 17).

To measure water depth in each environmental quadrat, a meter tape was attached to a floating buoy and a SCUBA diver extended the tape from the surface to the bottom. For sediment sampling, two sediment cores were collected within each environmental

quadrat and homogenized to form a composite sample. The corer was a custom-built 7.6-cm diameter PVC gravity corer, cut at an angle to pierce through dense vegetation (Kornijów and Kairesalo 2013). The top 10-cm of each core was extruded to represent sediment characteristics found in the rooting zone of submerged macrophytes. Sediment samples were stored in plastic bags and refrigerated before being sent to the University of Minnesota's Research and Analytical Laboratory for analysis.

The analytical laboratory analyzed composite sediment samples for total nitrogen (TN), total organic carbon (TOC), Bray phosphorus (BP), and texture. Sediment samples were prepared for analysis by drying under forced air at room temperature, followed by crushing and sieving through a 2.0-mm stainless steel sieve (Brown 1988). TN was assessed using a LECO FP-528 Nitrogen Analyzer (Yeomans and Bremner 1991; Lee, Nguyen, and Littlefield 1996). TOC was determined by dry combustion at 900°F and subsequent measurement of CO_2 evolution using an Elementar, Inc. VarioMAX C/N Analyzer (LECO Corporation). For the BP analysis, phosphorus was extracted by shaking air-dried soil with 0.025 M HCl and 0.03 M NH_4F for 5 minutes; BP was then determined by the molybdate-blue method using ascorbic acid as a reductant (Brown 1988).

Textural analysis was used to determine the approximate proportion of sand (50-2000 μm), silt (2.0-50 μm) and clay (<2.0 μm) particles in samples (Miller, Kotuby-Amacher, and Rodriguez 1997). Prepped soil was shaken for 16 hours with 5% sodium hexametaphosphate; the suspension was transferred to a sedimentation cylinder and shaken vigorously to re-suspend particles. An ASTM No. 152H hydrometer was used to

take two readings, one at 40 seconds and another at 6-8 hours; the percentage of sand, silt and clay in the soil was calculated from the resulting hydrometer readings. Unfortunately, the texture analysis used is designed for mineral-dominant soils (*Research Analytical Laboratory, pers. comm*) and some of the sediment samples were too rich in organic matter to be assessed for texture. Of 204 total samples, 56 could not be analyzed for texture: 8 from Koronis and 48 from Moose.

Statistical analyses

R statistical software version 3.4.1 was used to perform all analyses for this study (R Core Team 2017). Sediment data were modified prior to statistical analyses. TN and TOC were reported as proportions of nitrogen and organic carbon per sample and converted to percentages to aid interpretation. Texture was reported as three measures: percent silt, sand, and clay. Percent silt and clay were combined to form a composite 'non-sand' variable indicative of finer-textured sediments to evaluate the hypothesis that *Nitellopsis* prefers finer-textured, more organic-rich sediments. Additionally, percent silt, sand, and clay could not all be used as predictors because they were perfectly correlated (summing to 100%), which would be problematic for model fitting. Pearson correlation matrices were used to identify cursory relationships between *Nitellopsis* and environmental measures and to screen for collinearity between environmental predictors.

A generalized linear mixed effects model (GLMM) with a negative binomial error distribution was used to evaluate the relationship between *Nitellopsis* cover and the following environmental covariates: water depth, TOC, TN, percent non-sand, and BP. A negative binomial error distribution was suitable because *Nitellopsis* cover did not follow

the equal mean-variance assumption of the Poisson distribution (Booth et al. 2003). Lake ID was included as a random effect in this model to account for data collected within three individual study lakes. Since there was no *a priori* expectation for which variables might best explain *Nitellopsis* abundance, a maximal model with all was first constructed with all predictor variables and their interactions as fixed effects. Nested random effects of transect within lake were included. Collinearity between fixed effects was evaluated using the 'check_collinearity' function from the performance package (Ludecke et al. 2020); one variable that contributed to high multicollinearity (TC) was dropped. Next, model terms were iteratively dropped based on AIC comparisons and significance thresholds ($p < 0.05$). GLMM models were fit using the glmmTMB package (Brooks et al. 2017).

The GLMM indicated the explanatory power of the environmental covariates in explaining *Nitellopsis* abundance was low (see Results). To confirm this, I additionally performed an exploratory, regression tree analysis to ensure that no key relationships between environmental variables and *Nitellopsis* abundance were missed. Regression trees are useful in exploratory analyses because they have relaxed assumptions compared to standard regression models and can represent complex relationships and non-linearities between variables (Lewis 2000). Regression trees were fit using the rpart package (Therneau and Atkinson 2020). A Poisson regression tree was used because the response, *Nitellopsis* cover, is a discrete variable. The initial tree included all possible predictors – water depth, TOC, TN, percent non-sand, and BP. The tree was pruned using a cross-

validation approach to generate a tree size that minimized the complexity parameter (Therneau and Atkinson 2020).

A final effort to identify important covariates for *Nitellopsis* abundance was made by fitting a model that included biological factors in addition to the environmental ones identified through model selection. Native macrophyte richness and native macrophyte cover were included in this model.

Results

Water chemistry observed on transects was within typical ranges observed for *Nitellopsis* occurrences in other studies (Sleith et al. 2015, Midwood et al. 2016, Muthukrishnan et al. 2018b); see Table 1 for a summary of water chemistry data. Water chemistry within study lakes was fairly uniform, which supported the initial decision to measure these variables at a coarser scale (i.e., at the transect rather than individual quadrat level). Dissolved oxygen levels and pH were comparable between all three study lakes; however, conductivity in Koronis was over two times higher than that in Moose and Winnibigoshish. Across all lakes, water depth in environmental quadrats ranged from 0.6 – 4.9 m (mean ± S.D. = 2.3 ± 0.8 m). Substrate texture was comparable between Koronis and Moose, with mean % non-sand of 30.1 ± 8.2 and 27.0 ± 7.1, respectively. In contrast, Winnibigoshish had coarser, sandier substrate, with mean % non-sand of 17.6 ± 3.4. Sediment chemistry measures were comparable across study lakes and had narrow ranges overall. See Table 2 for a full summary of sediment data.

Based on initial examination of correlations, *Nitellopsis* cover was significantly correlated with only one variable, TN (Figure 1). The GLMM model selection approach resulted in a model with water depth, percent nitrogen, and percent non-sand as predictor variables (Table 3). All interactions were dropped following backwards AIC model selection. TOC was dropped due to high collinearity with other variables. BP was dropped because a majority of observations were below the detection level and removal of this variable did not worsen model performance. *Nitellopsis* cover declined with increasing percent nitrogen (Figure 2). This was the only significant relationship identified in the GLMM. Water depth and percent non-sand were not significantly associated with *Nitellopsis* cover.

Regression tree analysis initially resulted in a complex tree with repeatedly occurring variables and many splits (indicative of data with low explanatory power). The pruning process resulted in a tree with a single node and no splits; in other words, a tree model using the environmental variables to predict *Nitellopsis* cover could not be created.

Including biotic variables in the environmental GLMM did not lead to stronger conclusions. However, the addition of native richness and native cover to the model made water depth the only significant environmental predictor for *Nitellopsis,* rather than TN. Native richness did not have a significant effect on *Nitellopsis*. Native cover was associated with slight declines in *Nitellopsis* cover (expected based on the inverse relationship identified in Chapter 2). This model effectively rules out native macrophytes as having had a strong influence on abundance of *Nitellopsis*, which could have been an explanation for the lack of explanatory power for environmental variables. The model

with environmental variables only is used as the basis for discussion, as exploring *Nitellopsis*-environment relationships was the objective of this study and inclusion of the native macrophyte data did not enable stronger inferences in this regard.

Discussion

Considerable unknowns remain regarding the fine-scale environmental preferences of *Nitellopsis*. In general, *Nitellopsis* cover did not show a strong response to the environmental variables measured in this study, aside from an unexpected, modest negative response to increasing nitrogen. Broadly speaking, there are two likely explanations for these patterns: 1) limitations in study methodology failed to detect associations between *Nitellopsis* abundance and these environmental factors, or 2) *Nitellopsis'* environmental niche, for these factors, may be sufficiently broad that its abundance was not influenced across the gradients observed in this study.

A potential limitation of this study is that percent cover may be a poor abundance measure to detect fine-scale environmental preferences of *Nitellopsis*. This is because cover estimates do not account for the three-dimensional volume of *Nitellopsis*, i.e., two dense beds of *Nitellopsis* could have the same cover despite one being a meter tall and the other only centimeters tall. This limitation of cover values is consistent with my observations sampling different lakes. *Nitellopsis* in Winnibigoshish was notably shorter than *Nitellopsis* in Koronis and Moose. Winnibigoshish also had the sandiest substrate of the three lakes, consistent with the hypothesis that *Nitellopsis* would grow better in mucky or fine-textured sediments – like those in Koronis and Moose. Despite the lack of

nuisance growth in sandy Winnibigoshish, substrate composition had no significant effect on *Nitellopsis* abundance in this study. It is possible that had I used an abundance measure that was more reflective of nuisance growth, e.g., biomass or canopy height, I would have detected an effect of substrate type that was missed by cover alone. In retrospect, percent cover combined with a measurement of *Nitellopsis* height could have been an ideal way to rapidly assess *Nitellopsis* abundance and accurately capture nuisance growth. It should be noted that, while the limitations of cover as an abundance measure are important to consider, Kirkwood et al. (2020) did measure *Nitellopsis* biomass and had findings similar to my own, i.e., that environmental parameters had little explanatory power for *Nitellopsis* abundance.

Sediment texture was expected to be a strong indicator of *Nitellopsis* abundance initially, but this hypothesis was not supported by the data. However, it is important to note that 56 of 204 sediment samples could not be analyzed for texture due to high concentrations of organic matter. Sediments with high organic matter would comprise the softest, finest textured sediments in the study and the absence of these data is a strong limitation of this analysis. The relationship between texture and *Nitellopsis* could, however, be opposite of what I expected. A recent study suggested *Nitellopsis* was more prevalent in sandy, open habitats because most native macrophytes cannot establish on such nutrient-poor sediments, a limitation that charophytes can bypass by not relying heavily on nutrient acquisition from sediment (Ginn et al. 2021). The relationship between sediment texture and *Nitellopsis* warrants additional attention in future research.

Another potential limitation concerns the range of environmental variability across study lakes. The sampling design of this study intentionally captured variability in *Nitellopsis* abundance, and quadrats were well-distributed across low to high cover of *Nitellopsis* for all study lakes. However, the ranges for environmental variables measured in this study ended up being relatively narrow, with the exception of sediment texture. It could be that these environmental factors do influence *Nitellopsis* abundance, but this dataset did not capture a sufficiently wide gradient in these factors to detect such effects. Broader sampling within study lakes and/or sampling of additional lakes may have led to stronger inferences regarding the effects of these parameters on *Nitellopsis* abundance. In addition to *how* variables were measured, *which* variables were measured is also important in evaluating these findings.

Since there has been limited work on the fine-scale habitat preferences of *Nitellopsis*, the environmental parameters chosen in this study were quite general. For example, all forms of nitrogen were measured in aggregate rather than differentiating specific forms, such as nitrate or ammonia. However, identifying specific compounds in sediment samples is more costly and would have limited sampling extent. The environmental parameters selected in this study are, in principle, important for charophyte growth and should not be disregarded in future studies of *Nitellopsis*' environmental preferences, despite the limited effects detected here. However, there are additional variables not considered in this study that could affect *Nitellopsis* abundance, e.g., light availability, exposure, and water column nutrients. These should be incorporated in future studies of *Nitellopsis*' preferences.

A negative trend between *Nitellopsis* abundance and nitrogen was the sole relationship observed in this study. Interestingly, this relationship was also identified in the only other study addressing the influence of fine-scale environmental conditions on *Nitellopsis* abundance (Harrow-Lyle and Kirkwood 2020). It is difficult to conclude what the mechanism for this relationship might be because only total nitrogen was measured. Harrow-Lyle and Kirkwood (2020) noted the same limitation with their study. However, there are possible explanations for this pattern. Elevated ammonium levels can inhibit algal photosynthesis, while nitrate and nitrite promote algal growth (Diaz-Pulido and Mccook 2005). Thus, if ammonium were the predominant form of available nitrogen, the negative association with *Nitellopsis* would make biological sense, especially as there is evidence that *Nitellopsis* responds positively to nitrite and nitrate. Midwood et al (2016) found that *Nitellopsis* presence was associated with elevated nitrate-nitrite. And in a laboratory study, *Nitellopsis* grew better in a solution containing elevated nitrate—though other nutrients were also present at higher concentrations (Pokrzywinski et al. 2020). More information is needed to make conclusive statements on the relationship between *Nitellopsis* growth and nitrogen. Future work on environmental associations of *Nitellopsis* should evaluate individual forms of nitrogen in relation to its growth levels.

Another possibility is that the environmental tolerances of *Nitellopsis* could be broad enough that fine-scale environmental gradients do not strongly affect its abundance and growth. This is possible considering that, within its native range, it was identified as having the greatest habitat breadth and highest tolerance index among a broader suite of charophytes (Rey-Boissezon and Auderset Joye 2015). And it may be that the niche of

Nitellopsis has further expanded following its establishment in a new range (Broennimann et al. 2007). The apparent ability of *Nitellopsis* to reach nuisance growth levels across the range of environmental conditions observed in this study suggests a high degree of invasiveness, i.e., ability to overcome geographic and environmental barriers and achieve invasion success (Hui et al. 2011). This is concerning because it suggests *Nitellopsis* could become dominant under a variety of conditions, in turn increasing its potential for ecological impacts (Magee et al. 2010). A high degree of invasiveness elevates the threat *Nitellopsis* poses as an invasive species in Minnesota and elsewhere in North America. Once established, *Nitellopsis* can rapidly expand and become a dominant species (Chapter 2). The environment undoubtedly has some effect on *Nitellopsis* growth, but perhaps invasion severity at fine scales is more strongly influenced by other factors, such as time since invasion, disturbance, or ecological attributes of the recipient invaded community (Capers et al. 2007). These factors were not assessed in the present study.

The still unexplained variation in *Nitellopsis* abundance within and between infested lakes poses a challenge for resource managers responding to and managing *Nitellopsis* infestations. Management attention should focus where *Nitellopsis* is the most successful to minimize its impacts. However, there remains uncertainty on whether habitat conditions can be used an indicator of potential *Nitellopsis* growth levels. Therefore, prioritization of within-lake management and monitoring sites based on high environmental suitability for *Nitellopsis* is not currently possible. There is a solid understanding of lake-level parameters important for *Nitellopsis* establishment that can

and is being used to guide surveillance efforts; however, the same does not exist for fine-scale conditions important for *Nitellopsis* abundance.

Based on my observations, finer-textured sediment did seem to support greater growth of *Nitellopsis*. Sheltered areas of the littoral zone with soft sediments appear to be prime conditions for *Nitellopsis* to grow at nuisance levels. *Nitellopsis* was abundant across a range of water depths in this study, but nuisance growth in shallow areas is most problematic as it can create substantial recreational interference in addition to ecological impacts. It is important to recognize that, while *Nitellopsis* can exhibit excessively high growth levels characteristic of invasive species, it also occurred at low densities within infested lakes. This was particularly the case in Lake Winnibigoshish, where *Nitellopsis* was widespread in the littoral zone but not growing tall or forming dense pillows or surface mats, as observed in other infested lakes. There is much worry among lake associations and other stakeholders that *Nitellopsis* will completely take over any lake it establishes in. While some lakes have seen alarming expansion (notably Lake Koronis in Minnesota), this may be more an exception than the rule. It is important to remember that, similar to native species, invasive aquatic plants occur more often at low densities than high ones (Hansen et al. 2013). Monitoring infestations over time to see if some lakes, or areas within lakes, maintain *Nitellopsis* at low densities could provide insight into the limiting factors for *Nitellopsis* growth. As awareness and prevention efforts for *Nitellopsis* continue to increase, hopefully more infestations will be caught early when abundance levels can be effectively managed. *Nitellopsis* is a hardy invasive species that

is broadly competitive with a high abundance potential, characteristics that make it a high threat invasive species that should be prioritized.

Table 3.1 Summary of water chemistry data collected in study lakes.

	Dissolved Oxygen (mg/L)	Conductivity (μS/s)	pH
Koronis	8.0 ± 1.8 (3.7 - 10.8)	577.8 ± 106.9 (482.0 -734.3)	8.5 ± 0.2 (8.1 - 8.7)
Moose	9.0 ± 1.7 (6.5 -14.3)	219.5 ± 8.3 (194.2 - 230.5)	8.8 ± 0.3 (8.2 - 9.7)
Winnibigoshish	10.7 ± 2.4 (7.0 - 16.6)	255.2 ± 16.8 (240.7 - 291.0)	8.8 ± 0.2 (8.4 - 9.2)

Mean ± standard deviation shown, along with the range of observed values in parentheses.

Table 3.2 Summary of environmental data that was collected with *Nitellopsis* abundance in study lakes.

	Lake Koronis	Moose Lake	Lake Winnibigoshish
Water depth (m)	1.6 ± 0.5 (0.6 - 2.3)	2.6 ± 0.5 (2.0 - 3.5)	1.8 ± 0.7 (0.7 - 3.3)
Total nitrogen (%)	0.1 ± 0.1 (0.03-0.2)	0.2 ± 0.09 (0.04 - 0.3)	0.04 ± 0.1 (0.01 - 0.4)
Total organic carbon (%)	2.3 ± 1.5 (0.9 - 6.5)	2.7 ± 1.4 (0.6 - 4.9)	0.6 ± 0.8 (0.1 - 5.1)
Bray phosphorus	1 ± 0 (1 - 1)	1.6 ± 1.2 (1 - 4)	6.9 ± 19.0 (1 - 137)
Non-sand (%)	28.9 ± 9.2 (17.5 - 46.3)	23.9 ± 6.6 (15 - 31.2)	17.6 ± 3.4 (15 - 37.5)

Mean ± standard deviation shown, along with the range of observed values in parentheses.

Table 3.3 GLMM fit to evaluate associations between *Nitellopsis* cover and environmental conditions.

	Log-Mean	SE	z value	*P*
Intercept	1.51	0.25	6.11	< **0.001**
Water depth (m)	0.08	0.09	0.92	0.36
Non-sand (%)	-0.002	0.009	-0.30	0.77
Total nitrogen (%)	**-2.53**	**1.06**	**-2.38**	**0.01**

Model assumed a negative binomial error distribution with a log link function. Model response variable is *Nitellopsis* cover. Model coefficients and associated *p*-values are shown; coefficients are untransformed and on the log-scale. Non-sand is the sum of percent silt and percent clay. Environmental variables with a significant effect on *Nitellopsis* cover in bold text.

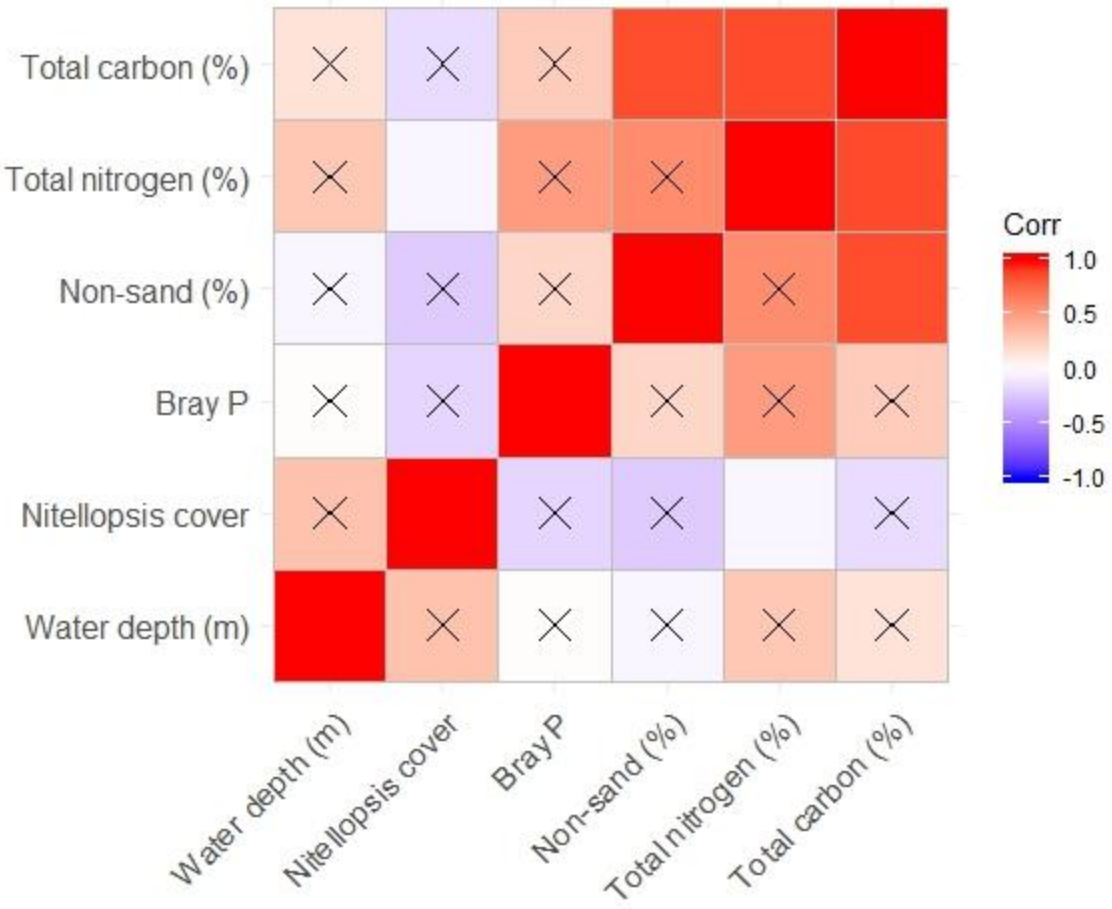

Figure 3.1 Pearson correlation matrix for environmental variables and *Nitellopsis* cover. 'X' indicates no significant correlation between variables. The only significant relationship found was between *Nitellopsis* cover and total nitrogen.

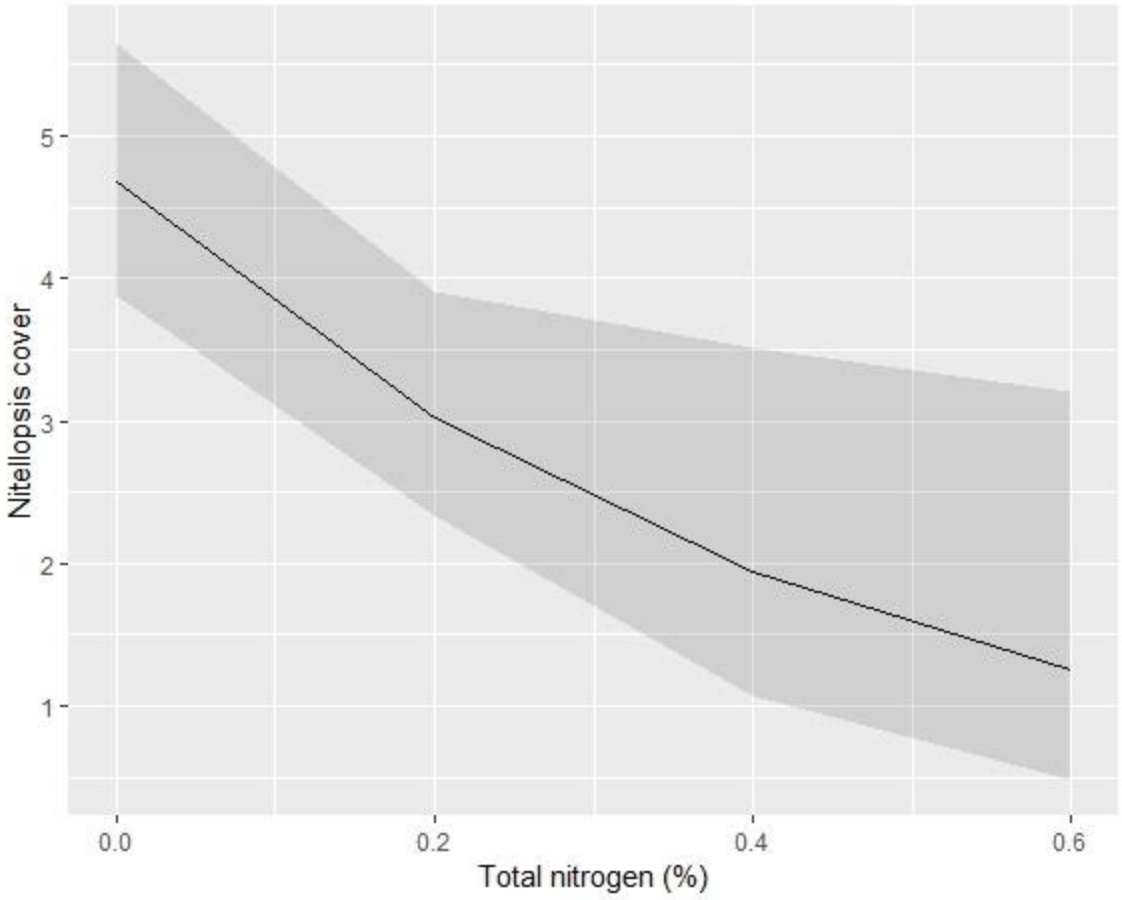

Figure 3.2 Relationship between *Nitellopsis* cover and sedimentary nitrogen. The solid line shows the marginal effects (with 95% confidence interval) of percent nitrogen from the final GLMM of the relationship between *Nitellopsis* and environmental variables.

Chapter 4

General Discussion and Conclusions

Aquatic invasive species (AIS) are a major conservation concern in freshwater systems because they can have pronounced effects on ecological communities and processes (Havel et al. 2015). Non-native aquatic plants are a particularly problematic group of AIS because they are easily spread between waterbodies (Cole et al. 2019) and can become highly dominant, displacing native species and interfering with human use of public waters (Ricciardi and MacIsaac 2011). Eradication and reversal of spread is rarely feasible for established populations of aquatic invasive plants (Hershner and Havens 2008). Thus, if spread prevention fails, long-term management to minimize impacts and limit further spread are typically the best available options. Prioritizing AIS based on their ecological impacts, and using knowledge of their biology and ecology to guide management responses, are critical for addressing the threat of AIS in aquatic systems (Simberloff et al. 2013).

My research provides urgently needed information on the ecological impacts and environmental associations of *Nitellopsis obtusa* (starry stonewort; hereafter *Nitellopsis*), an invasive macroalga that is rapidly emerging as a problematic invasive aquatic plant in the Great Lakes region (Larkin et al. 2018). Following extensive anecdotal claims of impacts to aquatic plants but limited published research, I conducted a comprehensive evaluation of *Nitellopsis'* interactions with aquatic plants and identified relationships between its abundance and plant community impacts. Invader abundance is often tightly related to impacts (Barney 2016), thus, I explored whether local environmental

conditions promoted nuisance growth of *Nitellopsis* with the goal of improving threat assessment and better anticipating impacts in invaded systems. This research advances knowledge of *Nitellopsis* ecology and biology, which to date has been insufficient for predicting and responding to the threat of this invader.

In Chapter 2, I used aquatic vegetation sampling to identify species- to community-level impacts of *Nitellopsis* on native macrophytes. I integrated multiple lines of evidence to draw robust conclusions on the ecological impacts of *Nitellopsis*. This included spatially extensive space-for-time-substitution sampling across three lakes and time sequence sampling that tracked *Nitellopsis* and native plants over a four-year period in two lakes. My results suggest that *Nitellopsis* has a high capacity to affect the diversity, abundance, and composition of native macrophytes and, importantly, that these impacts are closely associated with *Nitellopsis* abundance. The multi-year observations of *Nitellopsis* revealed that high interannual variability in its abundance made effects on native macrophytes fluid. However, where and when *Nitellopsis* became dominant, it had multiple impacts on native macrophyte diversity, structure, and composition.

In Chapter 3, I investigated environmental conditions associated with nuisance growth of *Nitellopsis*. I included environmental parameters expected to influence *Nitellopsis* abundance (water depth and sediment and water chemistry measures) and sampled in three lakes across widely varying *Nitellopsis* abundance. This investigation yielded limited results on the fine-scale environmental preferences of *Nitellopsis*, with only a slight negative relationship between sedimentary total nitrogen and *Nitellopsis* abundance being statistically significant. Nonetheless, there has been limited

investigation of fine-scale environmental attributes in *Nitellopsis*-invaded areas; my findings suggest that, like other invasive macrophytes, *Nitellopsis* may have broad environmental tolerances (Zedler and Kercher 2004). Alternatively, limitations of my cover-based method for measuring *Nitellopsis* abundance and/or of the environmental parameters that I measured may have obscured environmental preferences of *Nitellopsis*. More research is needed on factors responsible for growth of *Nitellopsis*, especially given the important relationship between abundance and impacts established in Chapter 2.

The ecological interactions and growth patterns of *Nitellopsis* presented here may not hold across its entire invaded range, which includes eight states and two Canadian provinces to date (Kipp et al. 2021). Conclusions in this study were drawn from a total of three lakes, which is a small subset of the 15 invaded lakes in Minnesota alone. Variation in lake characteristics and ecological communities could lead to different patterns of *Nitellopsis* growth and responses of native species to its invasion. Another caveat is that all results presented here are from unmanaged habitats. Control efforts may influence interactions between *Nitellopsis* and native macrophytes in ways that were not evaluated in the present study. Nonetheless, this study was designed to provide applicability to other systems through sampling of multiple study lakes and evaluation of impacts on general features, i.e., broad functional groups, of native macrophyte communities. Consistent abundance-impacts relationships were observed across the three study lakes and two sampling approaches, which provides support in generalizing to other systems.

Research on the biology and ecology of *Nitellopsis* should be prioritized given its potential for invasiveness and ecological impacts. Including my research, I know of only

two quantitative assessments of the plant-community impacts of *Nitellopsis* to date (Chapter 2 and Brainard & Schulz 2016). Expansion of this work, ideally in other regions of *Nitellopsis*' invaded range, is needed to examine whether impacts remain consistent between systems and in varying ecological communities. This is especially important given the frequently highlighted context dependency of invasion impacts (Stohlgren and Rejmánek 2014, Kumschick et al. 2015), which was reflected in results of my work (e.g., contrasting invasion patterns in Koronis and Moose on time sequence sampling transects). *Nitellopsis* has only recently emerged as a widespread invader in the upper Midwest and its invasion history in many lakes is short. Given this, it will be important to continue evaluations of *Nitellopsis'* impacts over time, as native communities may develop greater resilience to its invasion (Marchante et al. 2015) and/or *Nitellopsis* growth and spread may reach an equilibrium following rapid expansion during early stages of invasion (Fleming et al. 2014).

The fine-scale environmental preferences of *Nitellopsis* remain a critical research gap, especially given the mostly inconclusive results on this topic in Chapter 3. More investigation is needed to rule out the environmental parameters in this study as unimportant, and there are other water and sediment chemistry variables that have yet to be tested anywhere in the literature. The negative relationship between *Nitellopsis* and sedimentary total nitrogen perhaps warrants the most attention since Harrow-Lyle and Kirkwood (2020) and myself both identified this pattern. Specific forms of nitrogen should be evaluated since it is hard to infer the mechanism causing this relationship based on total nitrogen measures. It is possible that conditions in my Minnesota study lakes

were uniformly suitable for *Nitellopsis*, i.e., did not represent a wide enough gradient to detect dependence on these parameters. Stronger relationships might be revealed in habitat closer to the niche boundary of *Nitellopsis*, reinforcing the need for further investigation across its invaded range.

My research on the impacts and environmental tolerances of *Nitellopsis* emphasizes the importance of spread prevention efforts for this invader. *Nitellopsis* is the first invasive macroalga in the Midwest and arguably the only charophyte considered invasive globally. This novelty has led to high uncertainty of its threat and limited information on best management practices. The growing body of literature on *Nitellopsis* suggests it is a high threat invader (e.g., Chapter 2, (Brainard and Schulz 2016, Larkin et al. 2018)) that is also difficult to control (Glisson et al. 2018). Preventing further spread of *Nitellopsis* is critical for protecting aquatic ecosystems. Fortunately, there is still time for intervention because *Nitellopsis* is a recent invader that has not realized its full invasive range potential, both continentally (Escobar et al. 2016) and regionally (Muthukrishnan et al. 2018b). With strong messaging and outreach, preventing spread of *Nitellopsis* via boater-traffic has high chances of success as recent work has shown that this species is highly sensitive to desiccation (Glisson et al. 2020). I recommend that managers prioritize early-detection efforts in native charophyte dominated lakes because they may have high suitability for *Nitellopsis*, a close relative. Additionally, I recommend that prevention efforts (e.g., watercraft inspections) be prioritized for these lakes, because *Nitellopsis* is likely to have the most severe impacts in these systems.

When spread prevention fails and *Nitellopsis* becomes established, my research highlights the need for managers to implement long-term monitoring of both *Nitellopsis* and native macrophytes. The transects used in this study provided high resolution data on *Nitellopsis* and native macrophytes, but this method of data collection requires SCUBA diving and only covers small, localized areas. Establishing annual lake-wide monitoring using standard point-intercept surveys would be valuable in tracking *Nitellopsis* expansion and broad trends in the native macrophyte community over time. Identifying nuisance areas for *Nitellopsis* and targeting these for management is important to minimize impacts. My research indicated that *Nitellopsis* is able to displace native charophytes, likely through competitive superiority. In lakes where native charophytes are common, I recommend spending additional effort identifying particular charophyte species instead of lumping these species together, which is often common due to their cryptic identification. Using management to try to prevent the encroachment of *Nitellopsis* into charophyte meadows is strongly advised. Once *Nitellopsis* is mixed with native charophytes non-target impacts are a concern if copper algaecides are being used. My research showed that *Nitellopsis* abundance and expansion can be variable across space and time. Managers should recognize that *Nitellopsis* invasion can be fluid over time and lakes should not be viewed in binary as invaded or uninvaded. Monitoring *Nitellopsis* abundance and spread over time is important for developing response efforts and adapting management over time.

My findings did not provide the information to prioritize high-risk habitats for *Nitellopsis* growth. This would be valuable for management prioritization to pre-empt

nuisance growth and high impacts. Hopefully, future research will improve understanding of relationships between *Nitellopsis* abundance and local environmental conditions. Given the importance of abundance in driving impacts of *Nitellopsis*, I recommend resource managers spend extra effort monitoring areas of nuisance *Nitellopsis* growth during lake-wide monitoring, i.e., increase point density of surveys in these areas. Higher-resolution data on *Nitellopsis* abundance would be helpful in identifying highest priority areas for management. In general, data on fine-scale environmental conditions paired with *Nitellopsis* abundance are limited. Any contributions to this data are valuable for improving our understanding of *Nitellopsis* in its invaded range.

My research provides a substantial contribution to knowledge on the ecology and biology of *Nitellopsis* in its invaded range. Successful and appropriate invasive species management hinges on a thorough understanding of the target species. My research addresses uncertainty on the impacts of *Nitellopsis* and also highlights persistent knowledge gaps that should be prioritized in future work. *Nitellopsis* is a challenging invader that poses a substantial threat to inland lakes. Preventing widespread establishment of *Nitellopsis* and responding to new infestations to mitigate resource damage should be a top priority across Great Lakes states and provinces.

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